2011

RESTORATION OF TALL FESCUE PASTURES TO NATIVE WARM SEASON GRASSLANDS: DOES A FUNGAL ENDOPHYTE SYMBIOSIS PLAY A ROLE IN RESTORATION SUCCESS?

Sarah Lynn Hall
University of Kentucky, shall@chpl.net

ABSTRACT OF DISSERTATION

Sarah Lynn Hall

The Graduate School

University of Kentucky

2011

RESTORATION OF TALL FESCUE PASTURES TO NATIVE WARM SEASON GRASSLANDS: DOES A FUNGAL ENDOPHYTE SYMBIOSIS PLAY A ROLE IN RESTORATION SUCCESS?

ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Agriculture at the University of Kentucky

By
Sarah Lynn Hall

Lexington, Kentucky

Co-Directors: Dr. Rebecca McCulley, Assistant Professor
and Dr. Timothy Phillips, Associate Professor

Lexington, Kentucky

2011

ABSTRACT OF DISSERTATION

RESTORATION OF TALL FESCUE PASTURES TO NATIVE WARM SEASON GRASSLANDS: DOES A FUNGAL ENDOPHYTE SYMBIOSIS PLAY A ROLE IN RESTORATION SUCCESS?

Tall fescue, a cool-season grass native to Europe, central Asia, and northern Africa, has been widely distributed throughout the U.S. for use as turf and forage. Following its widespread planting, its ability to associate with a toxic fungal endophyte, *Neotyphodium coenophialum,* was discovered. Research has linked this fescue-endophyte association with increased biotic and abiotic stress resistance in endophyte-infected (E+) versus endophyte-free (E-) plants, and these differences may affect the ability of land managers to eradicate tall fescue and restore native grasslands. I conducted three studies to examine whether E+ tall fescue plants respond differently to management than E- plants, and whether the success of planted native species might be impacted via indirect soil effects. My overall hypotheses were that E+ plants would recover from restoration/eradication efforts better than E- plants, and that E+ fescue would reduce microbial symbionts in the soil needed by planted native species.

I first conducted a field study of a tall fescue pasture consisting of four sub-units being restored with different combinations of prescribed burns and/or herbicide applications, as well as an unmanaged control. I found no evidence of E+ plants preferentially surviving restoration management; however this field had unusually low endophyte infection rates to begin with. The second study was a greenhouse experiment in which I measured growth of E+ and E- plants exposed to different watering regimes (wet, dry) and prescribed burn treatments (none, one, or two burns). Watering regime significantly affected all measured growth parameters (wet>dry), but few endophyte effects were found and when present were opposite the hypothesis (E->E+). All burned plants quickly re-grew

tiller lengths comparable to the unburned control, with recovery occurring faster following the second burn compared to the first. My final study examined growth and arbuscular mycorrhizal colonization of native species planted by seed into soil from beneath E+ and E- tall fescue. I observed few differences in mycorrhizal colonization or biomass for seedlings between soil from E+ and E- tall fescue. Taken together, my results indicate endophyte status of tall fescue pastures being restored to native grassland species may not be important in governing restoration success.

KEYWORDS: Tall fescue, Endophyte, Grassland Restoration, Mycorrhizae, Prescribed Fire

Sarah L. Hall
Student's Signature

2 Aug 2011
Date

RESTORATION OF TALL FESCUE PASTURES TO NATIVE WARM SEASON GRASSLANDS: DOES A FUNGAL ENDOPHYTE SYMBIOSIS PLAY A ROLE IN RESTORATION SUCCESS?

By

Sarah Lynn Hall

Rebecca L. McCulley
Co-Director of Dissertation

Timothy D. Phillips
Co-Director of Dissertation

Charles T. Dougherty
Director of Graduate Studies

2 Aug 2011

RULES FOR USE OF DISSERTATIONS

Unpublished dissertations submitted for the Doctor's degree and deposited in the University of Kentucky Library are as a rule open for inspection, but are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but quotations or summaries of parts may be published only with the permission of the author, and with the usual scholarly acknowledgements.

Extensive copying or publication of the dissertation in whole or in part also requires the consent of the Dean of Graduate School of the University of Kentucky.

A library that borrows this dissertation for use by its patrons is expected to secure the signature of each user.

Name	Date

RESTORATION OF TALL FESCUE PASTURES TO NATIVE WARM SEASON GRASSLANDS: DOES A FUNGAL ENDOPHYTE SYMBIOSIS PLAY A ROLE IN RESTORATION SUCCESS?

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By

Sarah Lynn Hall

Lexington, Kentucky

Co-Directors: Dr. Rebecca McCulley, Assistant Professor
and Dr. Timothy Phillips, Associate Professor

Lexington, Kentucky

2011

ACKNOWLEDGEMENTS

The research included in this dissertation was supported by numerous people, without whom it would not have been possible. First and foremost I'd like to acknowledge my advisor, Dr. Rebecca McCulley, who provided guidance and mentorship throughout the project. During the first two and a half years of my project I was employed full-time at Kentucky State University. Without the support of KSU's Land Grant Program, and in particular my supervisor, Dr. Robert Barney, I would never have been able to pursue this project and degree. Support from KSU provided me with a unique opportunity to develop this research project in collaboration with Rebecca, while also pursuing separate research during that time. For the final year of my project, I was fortunate to receive support from a Kentucky Opportunity Fellowship from the UK Graduate School and the Center for Ecology, Evolution, and Behavior.

My committee members, Dr. Tim Phillips, Dr. Carol Baskin, and Dr. Mary Arthur provided helpful input during project development and feedback after the research was completed. Tim Phillips also provided support for the project by allowing me to use tall fescue and soil from his plots at the UK Research Farm, and Mary Arthur provided the heat-sensitive paint tags used in the burn study. The Kentucky State Nature Preserves Commission, and in particular Joyce Bender and Zeb Weese, supported this work by allowing us access to Crooked Creek State Nature Preserve for vegetation and soil sampling. They also provided access to many other preserves and allowed sampling of tall fescue for endophyte infection frequencies. Keith Clay at Indiana University allowed me to collect soil from his research plots in Bloomington, and Liz Koziol (from Jim Bever's lab at Indiana University) trained me in microscopal scoring of mycorrhizae. Mark Coyne provided use of a microscope to score slides for mycorrhizal colonization, and Tina Tillery of University of Kentucky Regulatory Services provided much of the endophyte testing for the project.

Help provided by the following people, in both the lab and the field were invaluable: Maria Almagro-Bonmati, Luke Box, Alex Breton, Glade Brosi, Elizabeth Carlisle, Allison Cooke, Jarred Earnest, Caroline Ketron, Ben Leffew, Payton May, Jim Nelson, Katie Routt, and Marvin Ruffner. Xia Yu provided statistical support.

And finally, for unending support throughout this process, I thank Jake Royse. I used to always think "What did *they* do?" when I would hear or read someone's thanks to their partner in the acknowledgements for a research project or degree. But I get it now. Thanks for all of it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter One

Introduction to Tall Fescue and Native Grassland Restoration

Natural History of Grasslands in Eastern North America & Kentucky

Grasslands are broadly defined as communities dominated by members of the Poaceae (with woody species largely absent) and are widespread throughout the world, covering approximately 40% of Earth's land cover (GRN Undated). Unlike prairies in the western U.S., which may often represent successional "climax" status due to water limitations, grasslands in the eastern U.S. have been considered "subclimax" successional stages, that largely depend on disturbance for their maintenance, without which they quickly succeed to forest (Weaver & Clements 1929). The presence of grassland in the central U.S. has long been explored by ecologists, with the earliest seminal work being Henry Gleason's "The Vegetational History of the Middle West" (1922). Edgar Transeau (1935) presented a vegetation distribution map of the so-called "prairie peninsula" in the eastern U.S. (Fig. 1.1), depicting the distribution of grassland in the region at the time of European settlement. He also presented a list of factors that should be included in any explanation of how and why the vegetation got established and persisted in these areas. These factors include the fact that the entire region was forested prior to the establishment of these prairies, that the dominant grasses could outcompete woody seedlings once established in the region, and that humans have played a role in the region for quite some time. Others have also explored the historical pattern of grassland cover in the eastern U.S. (e.g. Dicken 1935; Küchler 1964), and we now understand that grassland distribution in the region depends on a number of ecological variables, including severity of droughts, frequency of dry seasons, and both human and lightning-ignited fires (Chagnon et al. 2002). The degree to which each of these contributes to the

dominance of grassland vegetation at a given site varies, and other factors may be important as well, including soil properties and the presence of large mammalian grazers.

Within the state of Kentucky, grasslands are believed to have covered 6,000-10,000 km^2 at the time of European settlement (Jones 2005). They occurred primarily in the "Big Barrens Region" of west central Kentucky (Baskin et al. 1999), which appears as the isolated half-circle in Kentucky in Transeau's map (Fig. 1.1), and also corresponds with the Dripping Springs Escarpment geologically (USGS 1981). Clearly edaphic characteristics of this region play a role in maintaining grassland communities in this area today, with changes in underlying bedrock and soil depth driving differentiation of vegetation into deep soil barrens, xeric limestone prairies, and limestone cedar glades (Baskin & Baskin 1975; Lawless et al. 2004). Examination of pollen data from Jackson Pond in LaRue County indicated a large increase in grasses approximately 2,000 years BP (Wilkins et al. 1991), prior to which forested vegetation dominated. Historical accounts in written documents from the late 18th and early 19th century provide evidence of this region existing as grasslands when many European settlers arrived in the area (Baskin et al. 1994). Within this region, fire has undoubtedly played an important role in creating and maintaining grasslands, and is believed to have been used prior to European settlement by Native Americans (Baskin et al. 1999). In the central Bluegrass region of Kentucky, evidence of grass-dominated communities in the form of "woodland savanna" existed at the time of settlement (see Jones 2005 for a review; Fig 1.2). Recent evidence, however, suggests this region was mostly forested, with savanna openings created by removal of woody stems during and shortly following the first wave of European settlement, circa 1800 (McEwan & McCarthy 2008). Thus, the so-called "savannas" of the inner bluegrass region (Fig. 1.2) have a much more recent origin compared to the grasslands of the Big Barrens region.

Regardless of the specific conditions under which native grasslands originated in the eastern U.S. and within Kentucky, they have largely undergone drastic reductions within this range, as changes in disturbance regimes and land use have altered the distribution of this vegetation. Fire has clearly played an important role in maintenance of these systems throughout the region, and widespread fire suppression in the last 50 (Ruffner & Groninger 2006) to 100 (Augustine & Milchunas 2009) years has led to forest succession of many native grasslands. Agricultural use of areas once classified as native grasslands is widespread, with conversion to row crops and/or pasture responsible for huge losses in acreage (Barnes 2007). The removal and/or loss of mammalian herbivores that once played an important role in maintenance of the grasslands (DeSelm 1994), most notably bison (Samson & Knopf 1994), has eliminated the regular and widespread grazing history of the entire region, including Kentucky. Smaller mammals also serve important roles in grassland systems (Samson & Knopf 1994; Davidson et al. 2010) and have largely been reduced in number as well (Davidson et al. 2010). Finally, development of land (for houses, roads, shopping centers, etc.) has eliminated many patches of native grassland that remained, and invasion by non-native species has reduced species diversity of the remnant grasslands as well. Estimates of loss of native grasslands in North America as a whole since European settlement are as high as 99.9%, and in each state that historically had these communities, none to less than 1% of what remains is protected by public or private agencies (Samson & Knopf 1994). In Kentucky, no pre-settlement barrens exist today (Chester et al. 1997), and many of the high quality native grassland communities that do exist are xeric limestone prairies believed to have originated after European settlement (Baskin et al. 1994).

Where high quality native grasslands do exist in the eastern U.S., they are often highly prized for the ecosystem services they provide. Grasslands are

important habitat for a wide variety of animals, including insects (e.g. Kutschbach-Brohl et al. 2010; Vogel et al. 2010; Hall & Barney 2010), birds (e.g. Stanley 2010; Fisher & Davis 2011; Vos & Ribic 2011), and mammals (Grant & Birney 1979; e.g. Fuhlendorf et al. 2010; Richardson 2010). They also harbor a number of rare plant and animal species (Samson & Knopf 1994), serving as important reservoirs of biodiversity. Grasslands in Kentucky serve as home to 22 state-listed and two federally-listed (*Helianthus eggertii* and *Solidago shortii*) plant species (Jones 2005). Native grasslands can also be used effectively as rangelands managed for agricultural animal production (Masters et al. 1992; Sheley et al. 2006), and they sequester large amounts of carbon belowground (McCulley et al. 2005), which may help to offset some of the human-derived increases in atmospheric CO_2 concentrations. Increasingly, the value and benefits of native grasslands are being recognized, and land managers are working to restore areas no longer inhabited by native grassland species.

Grassland Restoration Efforts

In the state of Kentucky, the vegetative condition of areas targeted for native grassland restoration can vary significantly. Most often, land managers or farmers are starting off with pasture or cultivated fields. In the case of Kentucky's best current example of barrens vegetation (Ft. Campbell Military Reservation), at the time when the army took over the area and restoration began (1942), all non-forested land was being used for either row crops or as pasture. In order to achieve an open landscape for military operations, the army conducted periodic prescribed burns and/or bushhogging, which restored many native grassland species to the area (Chester et al. 1997). In other cases, land managers may be restoring grassland in areas that are at some successional gradient towards hardwood forest, which across the eastern U.S. typically begins with establishment of eastern redcedar (*Juniperus virginiana* L.). This early successional species has a number of impacts on native grassland flora (Rhoades & Shea 2003;

Limb et al. 2010) and soil characteristics (Rhoades & Shea 2003), and is often considered invasive in this context (Rhoades et al. 2005; Pierce & Reich 2010).

The term "restoration" has many different interpretations, but in my dissertation, I will use it to mean simply the establishment and/or maintenance of a desired plant community type. This is the same interpretation used by the Society for Ecological Restoration (2004), who define ecological restoration as "an intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability." The goals of native grassland restoration vary depending on the characteristics of a given site, but may include one or more of the following: eliminating woody stems/species, eliminating established non-native species, encouraging existing native grassland species, or establishing new native grassland species from seed. Common approaches to achieve these goals include the use of prescribed fire, mechanical removal of woody stems, selective herbicide use on woody stems, broad-spectrum or selective herbicide application on non-native grasses, and no-till seeding of native species (Washburn et al. 1999; Washburn et al. 2002; Barnes 2004; Harper et al. 2004; Barnes 2007).

Within the past 10-20 years, interest in grassland restoration has increased dramatically, accompanying the introduction of some federal cost-share (Farm Bill) programs aimed at improving soil and water quality providing assistance via grassland restoration. The Conservation Reserve Program (CRP) started as part of the 1985 Farm Bill, and has resulted in approximately 14.8 million ha of erodible cropland being planted to grassland species (Lindstrom et al. 1994), including 122,000 ha in Kentucky (Jones-Farrand et al. 2007). The state Natural Resource Conservation Service's WHIP (Wildlife Habitat Incentives Program) Program, which started in 1998, has resulted in over 4,100 ha of native grass/forb establishment and management in Kentucky (USDA NRCS 2006). Other programs that have provided assistance in grassland restoration (along with

other conservation measures) include the Grasslands Reserve Program (GRP) and the Landowner Incentive Program (LIP). These programs as a whole have clearly resulted in the alteration of land use in the U.S. and Kentucky, but a lack of ecological research to assess the outcomes of these programs has been identified (Kindscher & Tieszen 1998).

Federal agencies including the Bureau of Land Management, the U.S. Forest Service, the U.S. Fish and Wildlife Service, and the National Park Service have also increased their management efforts explicitly aimed at restoring the lands they manage to native grasslands (e.g. US Forest Service Interim Directive 2020-2010-1 "Ecological Restoration and Resilience"). In Kentucky, the "Oak-Grassland Restoration Demonstration Area Project" (LBL Undated) within the Land Between the Lakes is one such recent project developed to establish native grasslands with the use of different management strategies, including repeated prescribed fire as a cornerstone. In addition to federal agencies, numerous non-profits and state agencies take part in grassland restoration efforts in the U.S. Of note is The Nature Conservancy, which owns and manages land, but also helps private landowners achieve restoration goals by providing personnel and equipment to aid in restoration management, including federal and state cost-share programs. Within Kentucky, the State Nature Preserves Commission manages a number of grassland preserves, and the Department of Fish and Wildlife Resources manages a number of properties, and like The Nature Conservancy, also provides assistance to private landowners in the form of labor and equipment.

How Does Tall Fescue Factor into Native Grassland Restorations?

When pasture vegetation is targeted for native grassland restoration in the eastern U.S., tall fescue [*Schedonorus phoenix* (Scop.) Holub (syn. *Lolium arundinaceum* (Schreb. S.J. Darbyshire); *Festuca arundinacea* Schreb)] is very likely

the dominant plant species. Tall fescue is a non-native C_3 grass species, introduced in the late 1800's, which now covers 14 million ha in the United States, with its adapted range being the entire eastern U.S. and areas within the Pacific Northwest (Ball et al. 1993). The variety "Kentucky 31" is the predominant tall fescue in the landscape. This variety was collected in 1931 and released in 1942 and has been widely adopted and utilized as a forage, turfgrass, and for erosion control (Lacefield & Henning 1986; Henson & Safley 2009). This grass covers 2.2 million ha in Kentucky (Lacefield et al. 2003), including many areas once dominated by native grassland (e.g. Warfield Barren, Baskin et al. 1999). In 1973, the difference in animal performance between two adjacent tall fescue pastures in Georgia-- one causing problems for the cattle grazing it, and the other not – triggered extensive research as to the cause. It was discovered that a fungal endophyte was present at very high levels in plants of the pasture causing cattle problems, while it was present at low levels (~10%) in the pasture not causing cattle problems (Ball et al. 1993). This fungal endophyte, *Neotyphodium coenophialum* (Latch, Christensen and Samuels) Glenn, Bacon and Hanlin (syn. *Acremonium coenophialum* (Morgan-Jones and Gams); *Epichloë typhina* (Fries) Tulasne), was subsequently identified as the primary cause of a suite of problematic symptoms in livestock. This fungus produces several toxic alkaloid compounds that create symptoms such as rough hair coats, intolerance to heat, poor weight gain, fat necrosis, and gangrenous conditions in extremities for cattle (collectively termed "fescue toxicosis"), aborted fetuses, weak foals, or low milk production in mares, and low weight gain or milk production in sheep (Ball et al. 1993).

The genus *Neotyphodium* has been assigned to the asexual forms of fungi in the same group as *Epichloë*, which are commonly found in association with grasses (Schardl et al. 2004). *Neotyphodium* endophytes are considered more mutualistic than sexually reproductive fungal endophytes, and are thought to be

responsible for providing many benefits to host plants including anti-herbivory (insects & vertebrates) and anti-nematode properties, and increased drought tolerance and nutrient status (Schardl et al. 2004). The tall fescue-*N. coenophialum* symbiosis is considered mutualistic (Latch 1993), with the benefits of infection outweighing any physiological cost to the plant. Transmission of *N. coenophialum* is imperfect and vertical (mother plant-to- seeds), and infected host plants have been measured to yield seeds that are 81-91% infected depending on the maturity of the seed (Hill et al. 2005). In other grass species with *Neotyphodium* endophytes, transmission rates have been measured at 70-100% (Afkhami & Rudgers 2008), 88 and 96% (Gundel et al. 2009), and 68% (Canals et al. 2008). Due to the asexual lifecycle of *Neotyphodium*, long-term persistence and spread of the fungus relies on persistence and reproduction of its host plants. Thus factors that alter the competitive ability of endophyte-infected (E+) tall fescue compared to endophyte-free (E-) tall fescue could change the endophyte infection frequency (EIF) in a given stand over time.

When endophyte infection frequencies have been measured throughout the range of tall fescue in the United States (4,500 samples from 30 states), the average infection level was 60%, with a range of 0-100%, depending on the site or stand sampled (Ball et al. 1991). Ky-31, the most common variety found in the landscape, clearly has higher incidence of the fungal endophyte than other cultivars (Shelby & Dalrymple 1987). In Kentucky, researchers found 83% of fields tested had over 50% EIF, with 53% being over 80% infected (Lacefield & Henning 1986). Changes in EIF of a given stand over time may depend on the selective pressures applied, whether abiotic or biotic. Environmental differences between sites or over time may alter competitive interactions between E+ and E- tall fescue (Hill et al. 1998). Shelby and Dalrymple (1993) observed increases in EIF over a 12 year period for plots with four different EIFs, but mowing did not affect the trend. Gwinn et al. (1998) exposed fescue pastures of four different EIFs

(0, 25, 60, and 80%) to three levels of grazing pressure (low, medium, high) and observed increases in EIF between 1-38%, with the greatest increases for those pastures starting with intermediate EIF (20-60%) under high grazing pressure. In addition, plant genotype and fungal genotype may interact to impact the fitness of the symbiosis in any given plant or stand, with not all genotypes (plant or fungus) created equal (Latch 1993; Marks & Clay 1996; Buck et al. 1997; Assuero et al. 2000; Bayat et al. 2009; Rudgers et al. 2010). When tall fescue stands of differing EIF have been followed over time, E+ stands are typically more persistent and/or competitive than E- stands (Marks et al. 1991; Bouton et al. 2001; Franzleubbers & Stuedemann 2005) or those with so-called "novel" endophytes (fungal endophytes that do not produce the ergot alkaloids associated with livestock problems) (Hopkins & Alison 2006). Growth of the fungus within tall fescue tissue can vary seasonally, and minimal temperature for endophyte growth is greater than that of tall fescue host plants (Ju et al. 2006). Temperature has also been linked with alkaloid levels of E+ plants, with higher temperatures favoring production of these compounds (Latch 1993). This and other research has led to differences in genotype and endophyte recommendations for the eastern U.S. range of tall fescue use, with those areas in the southern-most regions encouraged to use E+ tall fescue, because E- stands persist poorly in these hot environments, but those in the middle and northern ranges are encouraged to use E- (Fig. 1.3).

Explorations into impacts of endophyte-infected tall fescue at the stand and regional scale have discovered significant alterations of community and ecosystem processes including nutrient cycling, soil microbial activity/composition, succession, herbivory, and interspecific competition. Higher soil organic carbon and nitrogen have been observed under E+ compared to E- tall fescue (Franzluebbers et al. 1999; Siegrist 2008) and high endophyte infection has been shown to alter soil microbial biomass and activity

(Franzluebbers et al. 1999; Van Hecke et al. 2005; Jenkins et al. 2006; Siegrist 2008; Buyer et al. 2011). However, these differences may be dependent on soil fertility (Franzluebbers & Stuedemann 2005) and cultivar (Franzluebbers 2006). Studies have also found alterations of decomposition rates, with slower rates for E+ litter compared to E- and for litter placed in E+ plots compared to E- (Lemons et al. 2005; Siegrist et al. 2010), although no endophyte effect was observed in a 60-day pot experiment (Lemons et al. 2005). Many nematodes are apparently negatively impacted by E+ tall fescue (Bernard et al. 1997), and composition of collembola differed between E+ and E- field plots (Lemons et al. 2005).

A number of studies have examined plant community dynamics and individual plant fitness traits between E+ and E- tall fescue. E+ plants have been found to: be larger than E- plants (Clay 1990; Hill et al. 1991; Hill et al. 1998; Assuero et al. 2006), flower earlier (Newman et al. 2003) and more frequently (Clay 1990), and to tolerate high temperatures (Marks & Clay 1996) and water stress (Arachavaleta et al. 1989; Bouton et al. 1993) better than E- plants. In addition, E+ fescue has been shown to suppress succession to forest (Rudgers et al. 2007), to support lower plant diversity (Rudgers et al. 2010; but see Spyreas et al. 2001), and to be more competitive than E- plants (Hill et al. 1991; Marks et al. 1991; Rudgers et al. 2005; but see Hill et al. 1998). And finally, some evidence exists that E+ tall fescue inhibits other species via allelopathy (Malinowski et al. 1999; Orr et al. 2005). Collectively, these studies and others paint a picture of tall fescue benefiting greatly from, and even owing in large part its success in persisting and spreading throughout the United States, to the presence of *Neotyphodium coenophialum*.

What Effect Might the Tall Fescue-*Neotyphodium* Symbiosis Have on Native Grassland Restoration?

The techniques and methods appropriate for restoration of tall fescue pastures to native grasslands have received a good deal of attention (Washburn

et al. 1999; Washburn et al. 2000; Madison et al. 2001; Rhoades et al. 2002; Washburn et al. 2002; Ruffner & Barnes 2004; Ruffner & Barnes 2010) in part because tall fescue pastures cover much of the land targeted for these types of restoration efforts, but the tall fescue symbiosis with *N. coenophialum* has been largely ignored in this work. Given the clear differences in growth of E+ and E- tall fescue, as well as differences in stand characteristics over time (observed both above- and belowground), responses of E+ and E- dominated tall fescue pastures to restoration management may be different. If they do differ, land managers might benefit by being able to target certain pastures for restoration based on their endophyte infection frequency.

Common restoration practices of prescribed burns and herbicide treatments may impose stressful conditions on tall fescue plants. If E+ plants are better able to resist eradication efforts and recover more quickly following these treatments, we would expect grassland restoration to favor E+ plants over time. In addition, if E+ tall fescue stands alter soil properties in ways that would limit establishment of native species, restoration outcomes might differ depending on the initial EIF of the stand. In instances where E+ stands were treated to kill the E+ plants, and replanted with E- seeds, reestablishment of E+ plants has been documented (Smith 1989; Defelice & Henning 1990; Tracy & Renne 2005), indicating E+ plant are able to recover and persist following eradication efforts. Herbicide resistance has been demonstrated in endophyte-infected Italian ryegass (Vila-Aiub et al. 2003), which is a closely related symbiosis to that of tall fescue-*Neotyhpodium*. Prescribed burns increase light levels and decrease soil moisture at the soil surface (Rhoades et al. 2002), conditions under which E+ tall fescue growth may be favored over E-.

This dissertation project was developed to explore the possible role that symbiosis with *N. coenophialum* might play during grassland restoration practices in tall fescue pastures. The three individual studies described herein were

designed and constructed in sequence, and resulted from prior or concurrent observations of variable native grassland restoration success across the state of Kentucky. The first two studies explored differences in vegetative recovery following restoration management of E+ and E- plants, and the third looked at establishment of native planted species in soil previously occupied by E+ and E- plants. With ample supply of tall fescue pastures across its range, this research was designed to provide results that could inform land managers of how best to select tall fescue pastures for grassland restoration.

Figures

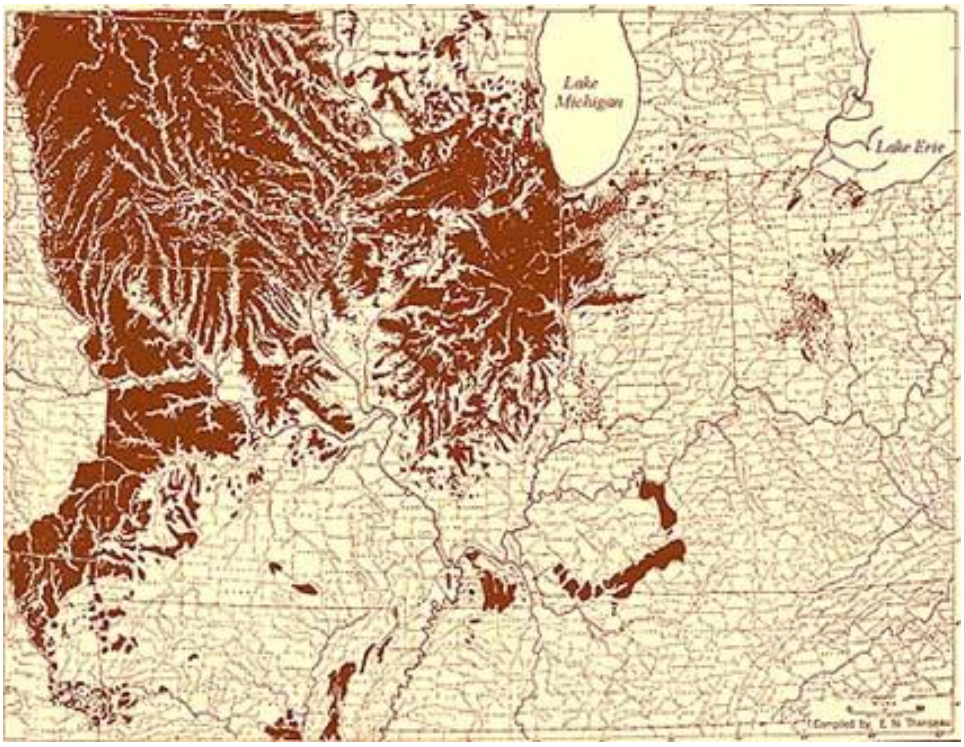


Figure 1.1. Transeau's map of the prairie peninsula. From: Transeau TA (1935) The Prairie Peninsula. Ecology 16: 423-441.

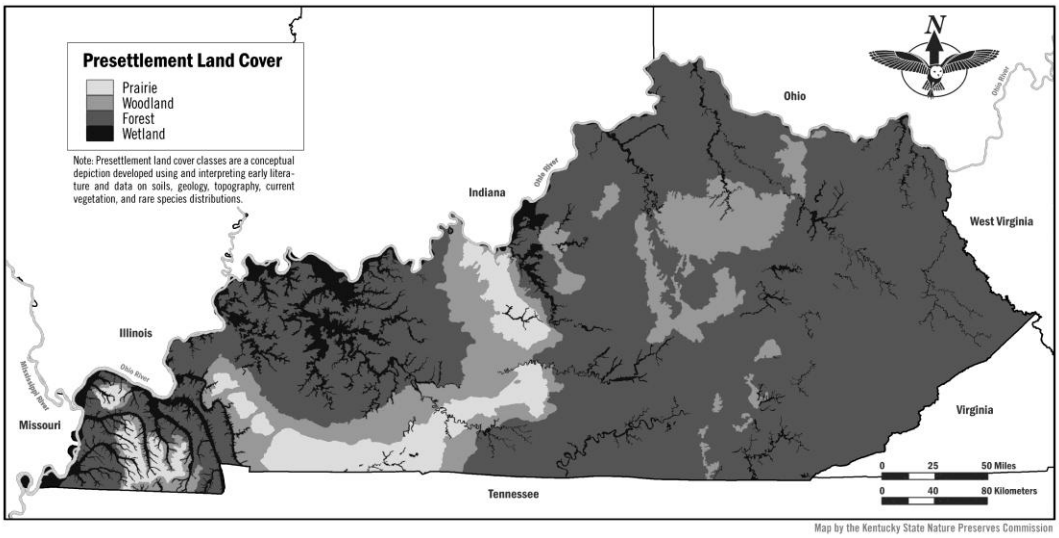


Figure 1.2. Vegetation cover types in Kentucky prior to European Settlement. Map done by the Kentucky State Nature Preserves Commission, Frankfort, KY.

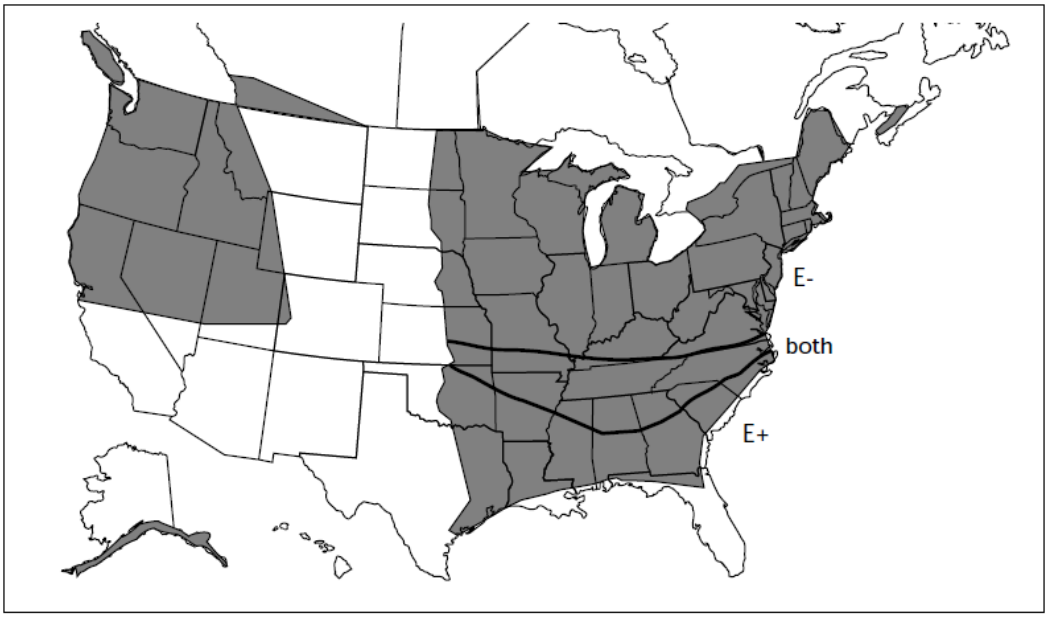


Figure 1.3. Adaptation and production areas of tall fescue in North America. Figure 7 in Oregon State University Extension Publication PNW-504 (April 2009), "Tall Fescue." Caption reads "Endophyte infection (E+) may improve survival in southern regions, but is not recommended in areas north of the transition zone (E- areas)."

Chapter Two

Restoration of Native Warm Season Grassland Species in a Tall Fescue Pasture Using Herbicide and Prescribed Fire[1]

Introduction

Over the last decade, ecologists have tried to identify common characteristics, traits, and mechanisms that may explain the invasive capabilities of certain species. Several hypotheses have resulted from this work. For example, invasive species may be more competitive at acquiring limiting resources (Rhazi et al. 2009); more able to exploit fluctuating resources during a period of high availability (Davis et al. 2000); and/or experience less pathogen, predator, and/or herbivore pressure in the new habitat (i.e., the enemy release hypothesis) that enables them to expend more of their energy on resource acquisition, growth, and reproduction (Torchin et al. 2003; Mitchell & Power 2003). Additionally, plant-soil interactions are frequently implicated in invasion success (Klironomos 2002; Callaway et al. 2004; Thorpe et al. 2009). These possible mechanisms are not mutually exclusive, and recent studies have documented that they may co-occur (Blumenthal 2005, 2006).

One mechanism by which some invading species may gain a competitive advantage over natives could be via microbial symbioses. Arbuscular mycorrhizal (AM) and non-AM soil fungi have been shown to facilitate invasive species spread and success (Klironomos 2002), but less is known about the potential role of aboveground fungal endophytes. For example, tall fescue, a non-native, invasive forage grass in North America (Clay & Holah 1999; Rudgers et al. 2010), is capable of forming a mutualistic association with the aboveground fungal endophyte, *Neotyphodium coenophialum* (Latch, Christensen and Samuels) Glenn, Bacon and Hanlin. This plant-fungal symbiosis has been shown to increase vigor (Latch 1993), fitness (Clay 1990), nutrient use efficiency (Cheplick et al. 1989), and drought tolerance (Arachevaleta et al.

[1]This manuscript with very minor differences is in press at the time of publication of this dissertation. Restoration of Native Warm Season Grassland Species in a Tall Fescue Pasture Using Prescribed Fire and Herbicides. S.L. Hall, R.L. McCulley and R.J. Barney. Restoration Ecology.
doi: 10.1111/j.1526-100X.2010.00749.x

1989; Hill et al. 1996), as well as resistance to mammalian herbivores (Rudgers et al. 2007; Rudgers et al. 2010) and insect pests (Siegel et al. 1990; Clay et al. 1993; Salminen et al. 2005), all of which may lead to endophyte-infected (E+) individuals being more competitive than endophyte-free (E-) tall fescue plants. In addition, some evidence has linked *N. coenophialum* presence to the reduction of AM spore abundance in soil (Chu-Chou et. al 1992; Antunes et al. 2008) and the inhibition of belowground AM colonization of other plants (Antunes et al. 2008). In greenhouse experiments, E+ tall fescue plants were more successful at invading diverse communities compared to E- plants (Rudgers et al. 2005), and in field plots, E+ plants were more persistent following grazing, mowing, and competition with bermudagrass than E- plants (Bouton et al. 2001). Clay and Holah (1999) found E+ tall fescue increased in cover and reduced diversity in large field plots over a four year period when compared to E- tall fescue plots, and growth of some tree species was reduced when grown in soil conditioned by E+ tall fescue (Rudgers & Orr 2009). Rudgers et al. (2010) showed both tall fescue host plant and endophyte genotypes can affect community level interactions. Limited evidence suggests that E+ tall fescue plants might recover better than E- plants following eradication efforts using herbicides (Smith 1989; Defelice & Henning 1990), and clear evidence of fungal endophytes conferring herbicide resistance has been demonstrated in Italian ryegrass (Vila-Aiub et al. 2003; but see Vila-Aiub & Ghersa 2001) and annual ryegrass (Kirkby et al. 2011). Collectively, these factors suggest that *N. coenophialum* might play a role in the ability of tall fescue to invade new areas and resist or recover following management and restoration practices such as fire, grazing, and herbicide applications. If restoration practices select for E+ tall fescue plants, then restoration of native species may be difficult to accomplish.

Grasslands once covered the entire central part of North America, but agriculture, urbanization, mineral exploration, fire suppression, and invasive species have largely altered these landscapes (Samson et al. 1998). Historically, pockets of grassland in the mesic-eastern United States were maintained by burning practices

employed by Native Americans (e.g. Baskin et al. 1999), as the humid nature of the environment would rapidly convert these areas to forest in the absence of repeated above-ground disturbance. At the time of European settlement in Kentucky, U.S.A., such grasslands covered 6-10,000 square kilometers within the state (Jones 2005). Quality "remnant" grasslands, which are home to a number of rare plants and insects, now cover less than 1 square kilometer in the state, as agriculture and fire suppression have resulted in widespread conversion of this landscape type to cropland/pasture, or they persist in a successional state to forest (Jones 2005). Tall fescue was planted widely across the southeastern U.S., including Kentucky, in the 1940's and 50's, for pasture. The current range of this species includes most of the eastern U.S. and parts of the Pacific Northwest, covering substantial acreage in eleven states (Ball et al. 1993).

Increasingly, concerns about potential negative effects of tall fescue on wildlife (Barnes et al. 1995; Madison et al. 2001) and livestock (Ball et al. 1993), as well as its invasive qualities (Rudgers et al. 2004; Rudgers et al. 2005) and proximity to extant, rare native grasslands, have inspired many land managers and preservation agencies to restore tall fescue dominated pastures to native warm season grasses and forbs. Replacement of cool-season pasture systems with native warm season grasses reduces the need for fertilizer, which may result in long-term economic benefits (Harper et al. 2004). In areas where a local seed source of native plants exists (either in the seed bank, seed rain, or from vegetative propagules), restoration efforts are aimed at eliminating tall fescue, while simultaneously encouraging native species without the introduction of commercial seed in plantings (as commercial seed is frequently not derived from local ecotypes). Application of grass-specific herbicides, as well as the use of prescribed fire (often in combination), are two main tools used to reach these restoration objectives (Harper et al. 2004). The short-term success of these efforts has been well-established (Washburn et al. 1999; Washburn et al. 2000; Madison et al. 2001; Rhoades et al. 2002; Barnes 2004); however, little information exists on the long-term outcome of repeated management practices on the plant communities. Furthermore, surveys of grassland

restorations across Kentucky suggest that these restoration techniques have variable effects on plant communities (Hall et al. unpublished data). It is possible that the degree of endophyte infection within the tall fescue population plays a role in the varying degree of success of these restoration efforts.

The primary objective of this study was to assess whether restoration practices maintained for >5 years successfully reduced tall fescue cover and increased native species cover and diversity. As part of this assessment, I determined: 1) whether vegetation composition and cover varied among units receiving different restoration practices; and 2) whether restoration efforts selected for E+ tall fescue plants, as would be indicated by higher endophyte infection rates in the tall fescue plants remaining following burning and/or herbicide application relative to an unmanaged control. My hypotheses were that restoration practices would be effective (as shown in short-term studies) in decreasing tall fescue cover while simultaneously increasing native species cover and diversity, and that restoration practices would reduce the number of E- plants, leading to higher endophyte infection frequencies within tall fescue remaining in the plant community following restoration.

Methods

Site Description

The location of this study was at Crooked Creek State Nature Preserve (CCSNP), a 294-ha preserve acquired by the Kentucky State Nature Preserves Commission (KSNPC) in 1999 and located in the northeastern part of the state, 3.9 km south of the Ohio river (38.646508˚N -83.577198˚W). The preserve includes areas of both high quality "remnant" xeric limestone prairie (Lawless et al. 2004), or glades (sensu Rhoades et al. 2005), and pastures planted to non-native forage grasses, dominated by tall fescue. The "Fescue Restoration Unit" contained a tall fescue pasture that has been actively managed for restoration goals with herbicide applications and prescribed burning since 1999. This 3.3-ha unit contained five adjacent sub-units (each 0.26-0.94ha)

that received different combinations of prescribed fire and herbicide applications, including an unmanaged control (Table 2.1). This experimental framework was used to compare plant composition and endophyte infection frequency in areas actively managed/restored to an unmanaged control. All herbicide applications used imazapic graminicide (Plateau [BASF]) applied with a boomsprayer on an all terrain vehicle, at either 293 or 585 ml/ha in early summer (J. Bender, KSNPC, pers. comm.). Prescribed fires were performed during the late winter/early spring, when native warm season grasses were still dormant (mid Feb-early Apr). We assumed that tall fescue was planted throughout this pasture sometime prior to the KSNPC acquisition. The topography and openness of the field suggested management practices prior to KSNPC ownership were similar over the entire area, and plant cover from the "control" sub-unit prior to the initiation of restoration treatments indicated tall fescue was the dominant graminoid ($\bar{x}$ =56% cover in 1999; KSNPC, unpublished data). Mowing and cattle grazing of the entire area occurred on a regular basis up until 1996. Soils within the restoration unit are silty clay loams.

Plant Community and Endophyte Infection Methods

I conducted vegetation surveys of all sub-units between 27 Sept and 4 Oct 2008. I established belt transects within each sub-unit, and visually estimated cover (to 1%) for all species within 1-m^2 quadrats placed on alternating sides of each transect. Because sub-units varied in size, transect lengths were established such that 1% of the total area for each was sampled, resulting in 27-94 1-m^2 quadrats (along 3-4 transects) per sub-unit. Transects ran approximately parallel within units.

Tall fescue tillers were sampled just outside the belt transects on both sides, with the number of tillers dependent on tall fescue dominance. I walked along each side of a belt transect scanning the ground for tall fescue, and cut vegetative tillers (no more than one per individual plant) at ground level using a razor blade as they appeared. For transects with very high tall fescue cover, I sampled a maximum of 2.5 tillers per meter

of transect (19-80 total tillers sampled per transect). Tillers were kept cool in transit from the field, double blotted onto nitrocellulose paper in the lab, and tested for *N. coenophialum* presence using Phytoscreen Immunoblot kits (Agrinostics, Watkinsville, GA). Endophyte infection frequency was determined by dividing the number of tillers testing positive for the presence of *N. coenophialum* by the total number of tillers tested per transect.

Statistical Analyses

Endophyte infection frequency and cover of common species (tall fescue; Japanese honeysuckle, *Lonicera japonica* Thunb.; little bluestem, *Schizachyrium scoparium* (Michx.) Nash; eastern redcedar, *Juniperus virginiana* L.), all exotic species combined, all native species combined, native forbs, native grasses/sedges, and native woody species were compared among the restoration treatments and the control using the means ANOVA procedure in JMP 7.0.2 (SAS Institute, Cary, NC), with transects serving as replicates within each sub-unit. For all cover estimates, means were first calculated on a square meter basis by averaging the quadrats in each transect, and then transect means were used to calculate each restoration treatment or control mean. For endophyte infection frequencies, the sub-unit mean was calculated using percent infection from each transect. Tukey-Kramer HSD was used to compare sub-unit means (α=0.05). Each species was classified as either native or exotic according to Jones (2005).

I used NMS (Nonmetric Multidimensional Scaling) ordination analysis on all species composition data by transect (mean cover of each species per m^2) with endophyte infection frequency overlaid as a second matrix to compare whole plant community structure among the restoration treatments and the control. Sorenson's distance measure was used and a Monte Carlo test evaluated the significance of axes compared to randomized data. Multi-response permutation procedures (MRPP) were used to compare each restoration treatment to the control in pairwise comparisons. MRPP generates a test statistic (p) for likelihood that observed differences are due to

chance, as well as a chance-corrected within group agreement statistic (A) which describes homogeneity within groups (max A=1 when all items are identical within a group). NMS, Monte Carlo, and MRPP analyses were conducted using PC-ORD 4.0 (McCune & Mefford 1999).

Because different numbers of 1-m^2 quadrats were sampled in different sub-units, which might bias measures of total species, I examined species richness across sub-units using species rarefaction curves with square meter quadrats as individual samples using EstimateS Win 8.0 (Colwell 2006). I calculated mean species richness per square meter and compared richness among the restoration treatments and the control using the same procedures as for cover values.

Results

Consistent with restoration goals, my results indicated an overall trend towards reductions in tall fescue cover and increases in native grassland species cover following five years of restoration management at this site. Although none of the four restoration treatments had significantly lower tall fescue cover compared to the control, mean fescue cover per square meter was low throughout the site, ranging from 1.1 – 17.9% across all sub-units. Sub-unit C (3 herbicide applications, 3 burns) had greater fescue cover than all other restoration treatments ($F_{[4,12]}$=5.82, p=0.008; Table 2.2), but was statistically similar to the control, despite having >2x more fescue cover than the control. Species rarefaction curves revealed this restoration treatment (sub-unit C) also had the lowest species richness (data not shown). Restoration goals of increasing native warm season grass dominance through the use of herbicide and fire were successful in achieving higher cover of native grasses ($F_{[4,12]}$=4.18, p=0.024; Fig. 2.1), which was primarily driven by an increase in little bluestem ($F_{[4,12]}$=3.82, p=0.030; Table 2.2), the most dominant grass found in native xeric limestone prairies (Lawless et al. 2006). Cover of native forbs also increased in response to restoration management ($F_{[4,12]}$=11.51, p<0.001; Fig. 2.1). However, restoration sub-units A, B, and D also had higher cover of Japanese honeysuckle ($F_{[4,12]}$=3.95, p=0.029; Table 2), an aggressive

invasive species (KY-EPPC 2008) which was almost absent in the control and restoration sub-unit C. All restoration treatments had lower overall species richness than the control ($F_{[4,12]}$=12.84, p<0.001; Table 2.2). Cover of woody species was greater in the control compared to all restoration treatments ($F_{[4,12]}$=84.95, p<0.001; Fig. 2.1). Eastern redcedar, the primary woody species of xeric limestone prairies and associated grassland communities (Lawless et al. 2006), was also greater in the control than any of the restoration units ($F_{[4,12]}$=5.36, p=0.01; Table 2.2), although cover of this woody species was relatively low in all sub-units.

The NMS ordination indicated a clear separation of the three transects within the control unit from transects in the restoration treatments. The ordination reached a 2-axis solution after 32 iterations with a final stress of 7.12 (Fig. 2.2), and cumulative r^2 for both axes was 95.5% (Monte Carlo test p=0.048 for both axes). Endophyte infection frequency did not appear significant in the overlay, which had a cutoff r^2 of 0.35. There was no clear separation between restoration treatments, suggesting there were no consistent differences in species composition among the combinations of prescribed burns or herbicide applications. When pairwise comparisons of transects within restoration treatments were compared to the control using MRPP, restored sub-units A and B were significantly different from the control (A=0.53, p=0.009 for sub-unit A, A=0.43, p=0.01 for sub-unit B). Restored sub-units A and B also had higher species richness (although not significant) than restored sub-units C or D (Table 2.2), and results from the species estimates (Jackknife 2 estimator) displayed the same patterns in species richness among sub-units (data not shown).

Contrary to my hypothesis, there were no significant differences in endophyte infection frequency between control and restored sub-units ($F_{[4,12]}$=1.16, p=0.376, Fig. 2.3). This result suggests that E+ tall fescue individuals were not preferentially surviving or re-colonizing the treated sub-units. Furthermore, there was no correlation between tall fescue cover and endophyte infection: the unit with the highest tall fescue cover (sub-unit C, 17.9% tall fescue) had the lowest infection rates (2.2$\pm$3.0; average $\pm$ 1

SE). Endophyte infection frequency, which was included in the second matrix of the NMS ordination, was not significantly correlated to either axis of the species composition data (Fig. 2.2), further suggesting that the presence or absence of *N. coenophialum* in tall fescue was not related to differences in vegetative composition across restoration treatments and the control.

Discussion

The primary effect of the cool-season pasture restoration efforts aimed at promoting native warm-season grassland was a shift in the dominance of the plant community composition from a non-native, forage plant - tall fescue - towards native grassland species. Restoration efforts resulted in desired increases in little bluestem cover and reduced tall fescue cover in three out of four restoration treatments, but these units also had greater cover of an undesirable species, Japanese honeysuckle. The presence of other invasive species following restoration management is of concern in a highly disturbance-dependent system, as management can often encourage additional invasive species (Rinella et al. 2009). In addition, restoration sub-units had lower species richness than the control, and percent cover of all native and exotic species did not differ between the restoration treatments and the control.

The highest species richness and lowest tall fescue cover occurred in the absence of burning or herbicide application (i.e., in the control). The control sub-unit has had very little disturbance since 1996, when cattle grazing and mowing were stopped at the site. Rudgers et al. (2007) showed that E- tall fescue stands rapidly succeed to forest in these mesic systems, if not managed to maintain a grassland structure (i.e., mowed; Foster et al. 2009). The low endophyte infection frequencies of tall fescue at our site (2.2 – 9.3% infected overall) may have facilitated rapid secondary succession in the undisturbed control sub-unit. Higher woody species cover and lower grass/sedge cover in the control support this supposition and suggest that the major differences in species composition observed across subunits at the site were driven by restoration practices impeding succession to forest. The ordination analyses successfully separated all

restoration units from the control, even one that had only received a single prescribed fire (sub-unit D). This suggests that increasing use of herbicides and burning does not have consistent effects on community composition and may not be warranted in all restorations. Aside from the clear restored vs. control separation, community composition was variable among transects within the restoration treatments, indicating a patchy mosaic not strongly reflecting treatment differences. Our results represent a single sampling event, aimed at capturing warm-season plant species. We acknowledge that we missed many cool-season species, but we believe the overall species richness trends and community composition differences presented here are likely reflective of overall patterns that would be observed year-round.

Restoration in cases where native seed or propagules are not introduced can present a number of challenges. Restoration practices that create disturbance may also create opportunities for the spread of additional problematic species. Facilitation of invasion by disturbance has been long recognized (e.g. Elton 1958), and restoration without introduction of undesirable species in restored communities may become increasingly difficult to obtain. For example, Kotanen (2004) found short-term increases in exotic species following soil disturbance in a coastal grassland (California, U.S.A.). Rinella et al. (2009) found herbicide applications used to control an exotic forb in a northern mixed grass prairie in Montana, U.S.A., were unsuccessful in reducing the target species sixteen years after application, and had lasting negative effects on native forbs. Conversely, Rice et al. (1997) found effective reduction of exotic species with no negative impacts on native forbs using the same herbicide as Rinella et al. (2009), and Blumenthal et al. (2005) reported that intensive disturbance-based restoration treatments (herbicide, prescribed burning, rototilling, and raking) resisted establishment of added weed seeds better than a non-disturbed seed addition only treatment. In mesic systems, some management/disturbance is clearly needed if grassland vegetation is desired, but the frequency of management may impact levels of

spread or invasion by other undesirable species. Balancing both the positive and negative impacts of restoration practices is a continuing challenge to land managers.

I found no increase in endophyte infection frequency of tall fescue in restoration treatments compared to the control, as all sub-units had low infection rates. These results are inconsistent with the findings of Smith (1989) and Defelice and Henning (1990), both of which suggested E+ plants recover better following herbicide than E- plants. I found, at least in one restored sub-unit (C), that tall fescue stands with low endophyte levels persist even after three herbicide applications specifically aimed at eliminating this species. Whether E+ plants resist eradication efforts better than E- plants may be environment dependent and requires further investigation. Assuming that pre-restoration levels of tall fescue in the control sub-unit (mean cover 56%) were representative of fescue cover across this site, reductions in tall fescue with very low E+ rates were observed in both restoration and control sub-units. Tall fescue persists under the same restoration management activities at higher cover levels in the region, and preliminary tests of 16 similar grassland restorations among seven nature preserves found that endophyte infection frequency of tall fescue ranged from 83–100%, with the average being 94% (Appendix 1). Endophyte infection frequencies can vary greatly across landscapes and regions, but low levels generally seem to be the exception. Of fescue populations sampled from 30 states, 90% had some level of infection and average endophyte infection frequency among these populations was 60% (Ball et al. 1991). Further, Lacefield and Henning (1986) reported 53% of tall fescue pastures tested in Kentucky contained infection levels >80%. In surveys of tall fescue in Illinois and its native range in England, Spyreas et al. (2001) found variable infection levels, with mean rates of 71% in Illinois samples, and 64% in those from England. Clearly, our study site was unique in low endophyte infection levels of tall fescue. Thus, it remains possible that endophyte infection frequency influences restoration success, with this site having overall low infection frequencies and relatively successful restoration (i.e., reduced tall fescue cover and increased dominance of native grasses). Given the widespread

distribution of tall fescue throughout much of the eastern United States, as well as its use as a forage worldwide, exploration of this potential relationship might lead to improved management and restoration approaches.

Tables

Table 2.1. Management history of five sub-units at Crooked Creek State Nature Preserve. H indicates herbicide application (see methods for chemical and quantity used) and B indicates prescribed burn within a given year (none occurred 2005-2008).

Sub-unit	1999	2000	2001	2002	2003	2004
A	B	H/B	B	H	--	B
B	B	H/B	H/B	H	--	B
C	--	H/B	H/B	H	--	B
D	--	--	--	--	--	B
Control	--	--	--	--	--	--

Table 2.2. Species richness and cover (mean m^2 ± 1 SE) of native and exotic species in the four restoration treatments (A, B, C, D – see Table 1 for descriptions) and an adjacent control. Means sharing the same letter were not significantly different between units within rows (α=0.05).

	A (2 H / 4 B)	B (3 H / 4 B)	C (3 H / 3 B)	D (0 H / 1 B)	Control (0 H / 0 B)
Species Richness	8.38(±0.3) c	10.93(±0.6) b	8.73(±0.3) bc	10.67(±1.1) bc	13.87(±0.3) a
Mean cover (%)					
Native Species	86.04(±6.5) a	85.62(±8.7) a	66.65(±6.0) a	80.14(±4.0) a	60.11(±4.1) a
Little bluestem	23.34(±5.6) ab	34.26(±4.1) a	32.10(±8.0) ab	35.46(±4.2) a	10.59(±0.9) b
Eastern redcedar	0.03(+0.0) b	0.02(+0.0) b	0(+0.0) b	0.06(+0.0) b	1.78(+1.5) a
Exotic Species	21.74(±7.2) a	9.0(±3.4) a	21.36(±5.8) a	7.14(±2.8) a	10.45(±3.7) a
Japanese honeysuckle	15.15(±4.7) a	6.20(±3.2) ab	0.05(±0.1) b	3.28(±2.3) ab	0.37(±0.2) b
Tall fescue	1.45(±0.7) b	1.12(±0.4) b	17.85(±6.3) a	1.21(±0.4) b	7.12(±3.9) ab

Figures

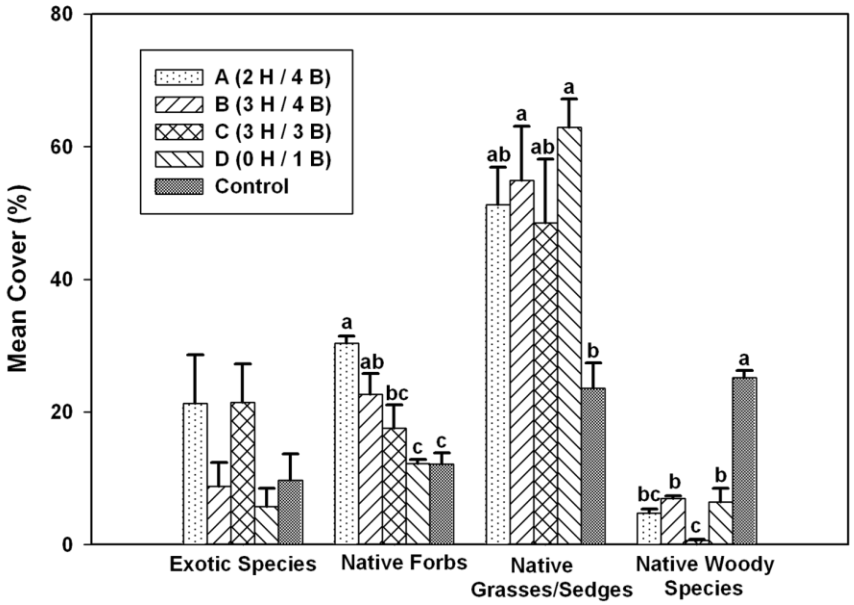


Figure 2.1. Mean cover (± 1 SE) of different plant groups in the four restoration treatments (A-D) and control. Number of herbicide treatments (H) and prescribed burns (B) indicated in parentheses for each sub-unit. Means accompanied by the same letter within a group were not significantly different (α=0.05). There was no significant difference across treatments for combined exotic species cover.

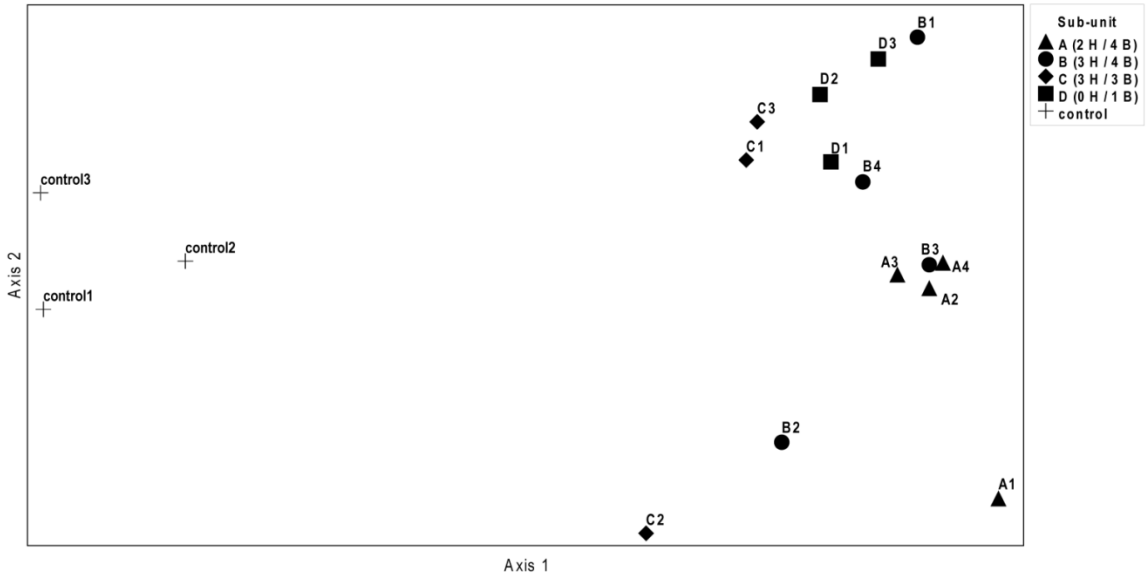


Figure 2.2. Biplot graph of the NMS ordination of vegetative cover data from 17 transects within five sub-units. Labels on graph indicate sub-unit (A, B, C, D, control) followed by the transect number (3-4 transects per unit). Average percent cover for each species per square meter of transect made up the main matrix, and endophyte infection frequency of tall fescue was included in the overlay analysis but was not significantly related to the observed patterns (cutoff r^2=0.35). The final stress for the ordination was 7.12, and cumulative r^2 was 95.5% (81.5% for axis 1, and 14.0% for axis 2). Monte Carlo tests indicated both axes were significant at p=0.048.

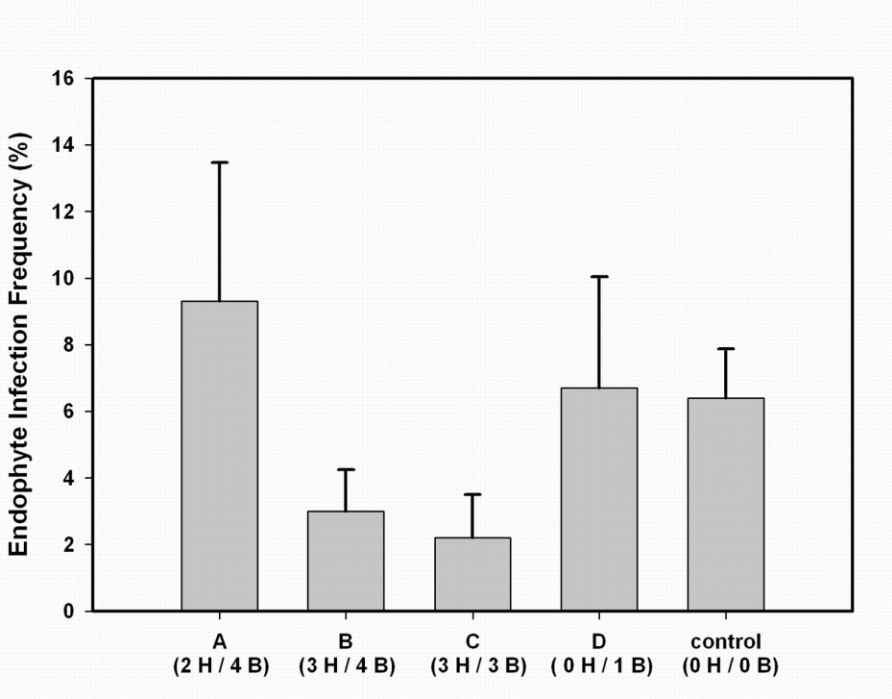


Figure 2.3. Mean (± 1 SE) tall fescue endophyte infection rates in the four restoration treatments (A-D) and control. Number of herbicide treatments (H) and prescribed burns (B) are presented in parentheses below each sub-unit on the x-axis. There were no significant differences between any sub-units (p=0.376).

Chapter Three

Growth Response of Endophyte-Infected and Endophyte-Free Tall Fescue to Prescribed Burn and Water Availability Treatments

Introduction

Prescribed fires are used widely in management of grasslands today (Ruffner & Groninger 2006). Within the mesic eastern U.S., prescribed fires serve primarily to reduce density/cover of woody stems (Taft 2003; Jenkins & Jenkins 2006; Duncan et al. 2008), which would otherwise increase until succession reached a forested state. Prescribed fire in this region is used not only to manage high quality remnant native grasslands, but it is also used as a tool to restore native grassland species to areas that have been planted with forage species and used as pasture. Within the latter context, fire may be used alone as a management tool, or it may be used in combination with herbicide application (Barnes 2004) to encourage native grassland species and discourage non-native species. In the case of planted pastures, the dominant plant species are typically non-native cool-season forage grasses, e.g. tall fescue in the transition zone of the eastern U.S. In a survey of four wildlife management areas in Kentucky, tall fescue was the dominant vegetation throughout the year (Barnes et al. 1995).

Tall fescue is frequently in symbiosis with the fungal endophyte, *Neotyphodium coenophialum*, and this symbiosis is known to increase tall fescue's stress tolerance over that of endophyte-free (E-) individuals (Bacon & Siegel 1988; Schardl et al. 2004). Endophyte presence within tall fescue populations can vary across the landscape: within a single field, some areas may have no individuals infected, whereas in other areas, all individuals present are infected. Extensive surveys show that on average endophyte infection frequencies (EIF) within tall fescue populations are typically >50% (Lacefield & Henning 1986; Ball et al. 1991). Surveys of 17 tall fescue pastures being

targeted for restoration across the state of Kentucky found all but one had EIF >80% (Appendix 1). Ky-31, the variety of tall fescue that is most common in pastures, has a higher occurrence of fungal endophyte symbiosis than other varieties (Shelby & Dalrymple 1987). The physiological benefits to tall fescue of hosting *N. coenophialum* are thought to be most pronounced under water (Arachevaleta et al. 1989; Assuero et al. 2000; Bayat et al. 2009) or nutrient stress (Malinowski et al. 1997; but see Cheplick et al. 1989), and the fungus may actually serve as a physiological drain or sink when the plant is not under such stress (Malinowski et al. 1997). Endophyte-infected (E+) fescue has been shown to have larger belowground biomass compared to E- tall fescue (Kelrick et al. 1990; Assuero et al. 2006), which could serve as a greater resource from which to recover following management activities that negatively impact aboveground growth of the plant. E+ plants have also been shown to respond to increased nutrient availability more than E- plants (Arachevaleta et al. 1989; Cheplick et al. 1989). It is possible that these endophyte-associated effects on tall fescue growth, physiology, and response to stress interact with the effects of prescribed fire to impact the effectiveness of this common grassland management tool.

Prescribed fire in mesic grasslands alters the abiotic environment, including increased light levels and decreased soil moisture at the surface, and increased nutrient availability (Rhoades et al. 2002). These abiotic effects may in turn affect biotic components of the grassland systems where they occur. Some rare plant species apparently benefit from the use of prescribed fire (Kaye et al. 2001; Gillespie & Allen 2004), and fire may serve as a recruitment tool in perennial grasslands (Zimmerman et al. 2008). Burning stimulates tillering of warm-season grasses (Cuomo et al. 1998), and it has been shown to reduce cover of some non-native species (Moyes et al. 2005; Abrams & Hulbert 1987; Towne & Kemp 2008) and C_3 species (Abrams & Hulbert 1987; Ansley & Castellano 2007; Towne & Kemp 2008). The behavior of fire (which determines impacts to the abiotic environment) can also be impacted by the vegetation present (Pyke et al. 2010), and areas dominated by C_3's, like tall fescue, often experience

reduced intensity of early spring burns (Taft 2003; Hall 2010), as they may have already begun to grow/green-up. This prior research then suggests that the effects of fire on tall fescue growth might be expected to be negative, positive, or neutral, depending on the effect of fire on the abiotic and biotic components of the system. Presence of the fungal endophyte within tall fescue might add another layer of complexity that could impact the effect of prescribed fire on tall fescue growth.

Previous research has concluded that tall fescue cover is not reduced with a single prescribed fire (Washburn et al. 1999; Washburn et al. 2000; Madison et al. 2001; Washburn et al. 2002; Barnes 2004), but the fungal endophyte status of tall fescue in these studies is unknown. Given that fire affects some of the same abiotic parameters also known to be important in determining whether endophyte presence within tall fescue increases or reduces its competitive ability (e.g., higher light, lower soil moisture, increased nutrient availability), prescribed fire may create a soil environment that favors E+ over E- tall fescue. If there are differences in the growth response of E+ and E- tall fescue to management with prescribed fire, this information could help inform management decisions by those wishing to restore native grasslands in tall fescue pastures.

I designed a controlled greenhouse experiment to test differences in growth of E+ and E- tall fescue following prescribed burn and water availability treatments. This experiment used established tall fescue plants (variety Ky-31, either with (E+) or without (E-) the common toxic *N. coenophialum*) to which I applied a water availability treatment, providing half the plants with adequate water supply ("wet"), and half the plants with half as much water ("dry"). I included an unburned control, a single burn treatment (1x), and a two burn treatment (2x). I hypothesized E+ plants would have higher biomass and growth compared to E- plants, and that differences would be most pronounced under the dry treatment. I also thought differences in biomass between the E+ and E- plants would be greatest for those plants that received the 2x burn treatment.

I hypothesized the total biomass of plants would be greatest for the 1x burn and lowest for the 2x burn, with the unburned control in the middle.

Methods

This experiment consisted of a full factorial design (2x3x2x6), with E- and E+ fescue, two burn treatments (1x, 2x) plus the unburned control, a wet and dry treatment, and 6 replicates per treatment combination.

Field Collection of Plant Material

On 6 and 9 March 2009, tall fescue plants were removed from 0.08-ha plots established in 2001 at the University of Kentucky Research Farm for the purpose of E+ and E- tall fescue seed production (n=2 plots of each endophyte status) (39.219167˚N - 86.541389˚W, see Fig. 4.2c for aerial view). Individual plants that had 2-3 overwintering tillers were selected from E- and E+ plots. These plots were dominated by tall fescue (visual cover estimates of 92% in E+ and 84% in E-) and had endophyte infection frequencies of 96% for E+ and 4% for E- (Siegrist et al. 2010). PVC pipe sections (7.5 cm in diameter, 22 cm in length) were placed around each individual plant, and a hammer was used to pound the pipe into the ground, leaving a 2-cm deep rim above the soil surface. Each pipe section was extracted, and contained the top 20-cm of soil and the individual tall fescue plant. These pipe sections served as pots in the greenhouse, and will be referred to as such. Each was marked as E- or E+, and plants were transferred to a greenhouse at the Kentucky State University Research Farm (Franklin Co.).

Greenhouse Conditions

Plants were given ambient light with a 21°C day (12hr) and 15.6°C night (12hr). Pots were individually numbered, and were randomly assigned burn treatment, watering regime, and replicate number. During this initial 2.5 week growing period (from 6 or 9 March to 25 March), all pots were watered twice a week to field capacity. Pots were arranged in six blocks across a single greenhouse bench. On 18 March, the

number of tillers for each individual pot was counted, and each was recorded as either large (with senesced material from the previous growing season surrounding new growth), medium (new growth from current year but with a fair amount of material), or small (new growth from current year with little material). All tillers were clipped to 4-cm ht, and clipped material was kept from each pot and placed in ziploc bags. The following day a wet weight was measured for all clipped material. Eight E- and eight E+ pots were randomly selected for harvesting at this time (18 March) to estimate belowground biomass prior to the experiment, and to obtain pre-treatment soil moisture levels. At the time of harvest, it had been two days since the last watering event.

Burn Treatments, Watering Regime & Fertilization

Senesced plant litter of native warm season grasses (primarily switchgrass and little bluestem) was collected from a field at the KSU Research Farm to serve as fuel for the prescribed burn treatments. It was placed in the greenhouse for one week to dry, and was then cut into approximately 3-cm pieces. Fire behavior is complex, and relies on a number of biotic and abiotic factors which result in variability across the landscape (Pyke et al. 2010), and could result in variability in fire behavior between different pots. To evaluate heat levels of the prescribed fire treatment, I made aluminum tags that were painted with different heat-sensitive paints (Tempil Inc., S. Plainfield, NJ) that change appearance at 79, 163, 246, 316, 399, and 510˚C (Fig. 3.1b). Each tag was wrapped in aluminum foil (which melts at 644˚C).

On 25 March, the first prescribed burn treatment was applied to all pots assigned to one of the two burn treatments (1x or 2x). A single heat-sensitive paint tag (that had all six paints) was anchored at the crown level of each pot with a paper clip (Fig. 3.1a). Pots were burned in (random) groups of 12 on a concrete floor inside the headhouse. Doors were opened on either side of the headhouse to allow for some air movement. Pots were placed such that paint tags were all in the same direction (with air flow). A

250ml cup was used to scoop dried native grass litter onto each pot. Care was taken to get as much litter as possible over each pot. A standard lighter was used to light the native grass litter in all pots for each group. Once the fire had burned out, paint tags were removed and marked with their respective pot number. It was raining outside the day of the first burn, and a sling psychrometer indicated relative humidity inside the headhouse to be at 100%. Ten subsamples of the native grass litter (250-ml each) were taken to calculate fuel moisture and to estimate variance in fuel amounts between pots. These were stored in sealed plastic bags to maintain the moisture levels they had at the time of the burn.

Water regime treatments began the day of the first burn, with only the wet pots receiving water immediately following the burn. On 1 April, both the wet and dry treatments were watered, the wet pots receiving 116ml (volume of water based on long-term average of weekly March-June precipitation for Lexington, KY, (Weather Channel Undated) calculated and applied based on the area of each pot) and the dry pots received half this amount, 58ml. Pots were watered with these amounts 2-3 times per week as needed for the rest of the experiment. On 8 May, all pots were fertilized with 58ml 20-10-20 fertilizer (Peters 20-10-20 Greenhouse Fertilizer Peat-lite). Wet pots were given an additional 58ml water without fertilizer to maintain this treatment. The same procedure was also used on 3 June to fertilize all pots.

On 12 May, the second prescribed burn treatment was applied to those pots assigned to that treatment. The same procedure was followed as for the first burn, except no fuel samples were taken for mass and moisture evaluations and no paint tags were used. Relative humidity was approximately 55% at the time of the burn. Prior to burning, all plastic markers used to identify and track individual tillers (see Growth Measurements, below) were removed, and tillers that emerged after the burn treatment were marked anew (as a new "cohort").

Growth Measurements

All tillers were individually marked in each pot using different colored strands of plastic (approx. 2-mm wide, 8-cm long) and measured weekly (during the first month) and bi-weekly thereafter. Individual tillers were measured by recording the distance from the base of the tiller to the longest green part of a leaf sheath on that tiller. New tillers that emerged during the experiment were marked and measured as they appeared. Visible signs of plant pathogens or insects pests were noted during measuring. Reproductive tillers were clipped to prevent seed from developing, and date of flowering was noted (these measurements occurred during the more frequent watering events). This clipped material was kept to be added to oven-dry aboveground material for biomass measurements.

On 26 Jun (100 days after experiment initiation), all control pots were harvested. Each tiller was cut at the soil surface and placed in a coin envelope. Tillers were stored cool, and double blotted onto nitrocellulose paper for endophyte testing (Agrinostics immunoblot assay). Soil from each pot was sieved, and roots were removed by hand-picking. Individual tiller material and pot root material were dried at 55°C for 48 hours to obtain biomass. A 5-g subsample of soil from each pot was used to measure gravimetric soil water content. Burned pots were harvested 10 Jul (2x burned) and 13 Jul (1x burned) (114-117 days after experiment initiation, and 107-110 days after the first prescribed burn), with the same procedures followed as described for the control pots. Weights of all crown and root material were ash-corrected by placing a 0.5-g subsample of harvested biomass in a muffle furnace at 550˚C for 4 hrs.

Statistical Analyses

Data were analyzed at the pot level for the following response variables using Proc GLM to test for effects of endophyte presence, watering regime, burn treatment, and all interactions: final total pot tiller length, number new tillers (difference between tiller number on 18 March, prior to treatment implementation, and at harvest), number

reproductive tillers, oven-dry total tiller biomass, oven-dry root biomass, and total oven-dry biomass (tillers, crowns and roots). LS Means procedure for pairwise comparisons of means (SAS 9.2, Cary, NC) was used to detect for significant differences between means. Means and standard errors were obtained using the Means and Standard Deviation procedure in JMP 9.0 (SAS Institute, Cary, NC).

In order to test whether the temperature of the first burn treatment affected growth, Proc GLM was used to test for the effects of burn temperature and its interactions with water regime and endophyte presence, on the total tiller length, number new tillers, number reproductive tillers, and mean length per tiller (total pot tiller length divided by number tillers present) for burned pots as measured just prior to the second burn (12 May) for those pots assigned to the 1x or 2x burn treatment.

To observe trends in growth for tall fescue tillers over the entire experiment, mean length per tiller ($\pm$ 1 S.E.) was calculated at each of the nine measurement intervals (which took place one to three days apart between treatments) and plotted on a line graph over time. Means ANOVA procedure and Tukey-Kramer HSD were used in JMP 9.0 to test for significant differences between Wet/Dry, E+/E-, and 1x/2x/unburned controls at each of these nine measurement intervals. Growth rates were also calculated for the different measurement intervals by dividing the difference in total tiller length by the number of days between measurements to get the total tiller growth rate (cm/day). For these calculations, lengths of tillers were assumed to be zero immediately following a prescribed burn. Means ANOVA procedure and Tukey-Kramer HSD were used in JMP 9.0 to test for significant differences within measurement intervals between burn treatments.

Results

Endophyte Infection Frequency and Initial Root Biomass & Soil Moisture

Endophyte tests of tillers harvested at the end of the experiment revealed twelve of 70 pots that were not 0% (E-) or 100% (E+) endophyte-infected. Of these twelve, only

two pots had endophyte infection frequencies (EIF= total number tillers testing positive/total number tillers) more than 50% different from what they were supposed to be (one E+ pot with 40% EIF, and one E+ pot with 28.6% EIF). These two pots were removed from the dataset to ensure statistical analyses were conducted on measurements from pots dominated by tall fescue of the correct endophyte status.

For the 16 randomly selected pots that were harvested prior to the implementation of water regime and burn treatments (18 March), soil moisture was significantly higher (p=0.0385) for the E+ pots (17.44$\pm$1.57%) compared to E- pots (13.63$\pm$0.55%). E- plants were extracted from the field three days earlier than E+ plants, but all had been watered to field capacity two days prior to harvesting, so it seems unlikely that differences in soil moisture were due to extraction date differences. Biomass of belowground material for these 16 pots harvested prior to implementation of the treatments revealed significantly (p=0.0499) higher root biomass for E+ (0.98$\pm$0.15g) compared to E- (0.63$\pm$0.06g). The wet weight of the plant material clipped and removed at this time (any material > 4-cm tall) for these same pots was not significantly different between E+ and E- plants (p=0.4617). Therefore, endophyte-related differences prior to the initiation of the treatments were only apparent belowground (root biomass and soil moisture).

Effects of Prescribed Burn Treatments on Growth

The heat-sensitive paint tags used during the first prescribed burn revealed that fire created temperatures ranging from <79˚C (no paints melted) to >316˚C but <399˚C (the fourth paint melted). Of the 48 pots that were burned, five were <79˚C (Fig. 3.1b), twenty-three were >79˚C but <163˚C (Fig. 3.1c), one was >163˚C but <246˚C (Fig. 3.1d), fifteen were >246˚C but <316˚C (Fig. 3.1e), and four were >316˚C but < 399˚C (Fig. 3.1f). Because the number of replicates was low in several burn temperature categories, pots were binned into those that had experienced fire temperature of <162˚C and those that had experienced fire temperatures of 246-398˚C in order to allow for LS Means

comparisons (the one pot that was >163˚C but <246˚C was removed from the dataset). Burn temperature did not have a significant effect for any of the measured variables using this binned Proc GLM approach. Given the variability of temperatures within each of these ranges, I also ran linear regressions to see if any of the growth variables might be significantly correlated to fire temperature. Again, no significant relationships were identified. This lack of burn temperature effect suggests that the variability observed in fire temperature at the crown level did not result in differences in tall fescue growth as measured in a greenhouse for 48 days after the burn treatment; however, additional replicates would help further assess this claim. Fuel samples from the first burn ranged from 4.15-6.54g (average 5.28$\pm$0.23) and fuel moisture ranged from 9.73-9.98% (average 9.88$\pm$0.0003). Surprisingly, burn treatment (1x, 2x, or control/no burn) did not significantly affect soil moisture averaged across wet/dry treatments, which was similar (10.4+0.5% for 1x, 10.8+0.5% for 2x, and 11.4+0.4% for control) in soils at the end of the experiment across burn treatments.

Burn treatment had a significant effect on total tiller length, number of reproductive tillers, tiller biomass, and total biomass as measured at the end of the entire experiment period (Table 3.1). The once and twice burned pots had greater total tiller length than the unburned control at the final harvest, while the opposite was true for number of reproductive tillers. The control had more reproductive tillers than either of the burn treatments (Table 3.2). Tiller biomass was greatest for the 1x burn treatment, intermediate for the control, and lowest for the 2x burn treatment (Table 3.2), but there was no effect of burning on root biomass (Table 3.1). Total biomass was greater for the control and the 1x burn treatment compared to the 2x burn treatment (Table 3.2).

When trends in total pot tiller length were compared over time, they varied by burn treatment for the first five measurement intervals following the 25 March prescribed burn (Fig. 3.2). At each of the first four measurement intervals during this period, the control pots had greater tiller length compared to the burned pots, but for the last measurement during this period (12 May, just prior to the second burn), the 1x

burn pots remained lower than the controls, but the 2x burn pots had become similar to the control (despite the fact that 1x and 2x burn treatments were the same at this point). By 26 May, two weeks after the second burn was performed on the 2x pots, there was no significant difference in total tiller length between either of the burn treatments. Burn treatment did not have a significant effect on total pot tiller length throughout the rest of the experiment.

When total tiller growth rates were calculated over the experiment by burn treatment, a number of trends emerged. Immediately following the first prescribed burn, growth rates for burned pots were depressed compared to the controls (for the two weeks following the burn), but by the next measurement interval (third week after the burn) the burned pots had significantly higher growth rates compared to the control (Fig. 3.3). At the fourth week after the burn these differences no longer appeared, and all growth rates were similar. Similar growth rates persisted until the second burn was applied to the 2x pots. Burning a second time stimulated higher tiller growth rates in 2x pots than 1x pots for the month following the second burn (Fig. 3.3). For the two last measurement intervals (mid to late June for all treatments, and late Jun to mid July for the 1x, 2x burned pots) there were no significant differences in growth rates.

Two pots had no aboveground live material at the final harvest- both were E-pots under the dry water regime that were burned once or twice. One of these had no aboveground material at the first measurement following the first prescribed burn, and the other had very low growth following the first burn that declined over time (no material present when second burn was applied). Given the low number of pots that experienced mortality, and the fact that one of them did have growth following the first prescribed burn, no conclusions can be made as to why these plants experienced mortality. The higher total tiller length at the final harvest for burned pots compared to the controls (Table 3.2) clearly suggests that burning did not negatively impact tall fescue growth, even when applied twice in a single season. Fire did depress growth

initially following the first burn, but then stimulated it, and no depression in growth appeared following the second burn (Fig. 3.3).

Effects of Water Regime on Growth

Water regime had the most pronounced and widespread effects on measured growth variables, being significant for all parameters (Table 3.1). In all cases, the dry watering regime had significantly lower measured growth responses at the final harvest than the wet treatment (Table 3.3). This was also true for the total tiller length at all measurement intervals, the dry pots had lower tiller length than the wet (data not shown). Water regime was the only treatment that significantly affected root biomass and new tiller number. A total of 29 reproductive tillers appeared in 24 pots over the course of the experiment, and all were in May. The 'wet' treatment had 2x the number of reproductive tillers than 'dry' (Table 3.3). Water regime significantly affected date of flower during May (p=0.0129), with plants under the wet treatment flowering earlier (on average, 'wet' plants flowered on May 6$\pm$2 days) than those under the dry treatment (average 'dry' date of flowering May 12$\pm$2 days). Clearly, tall fescue in those pots under the dry water regime was limited in growth compared to those under the wet water regime, as intended. Water regime had a significant effect on soil moisture of the pots at the end of the experiment, with the dry pots having significantly lower soil moisture than the wet pots (p=0.0003; 9.99+0.39% (dry) vs. 11.38+0.29% (wet)).

Effects of Endophyte Presence on Growth

Endophyte status significantly affected total tiller length and final tiller biomass (Table 3.1). In both cases, the E- tall fescue plants had greater growth than E+ (Table 3.4). Surprisingly, I did not find any significant interactions between the watering regime and endophyte presence or burn treatment (Table 3.1). The difference in soil moisture between E+ and E- pots observed at the initial harvest prior to implementation of the experimental treatments was no longer present at the end of the experiment (p=0.3865), indicating that effects of the water regime treatment on soil moisture had

over-ridden any differences present at the beginning of the experiment related to endophyte presence.

Discussion

Of the different treatments imposed during this experiment (endophyte status, water regime, burn), water regime had the most pronounced and consistent effect on tall fescue growth, with those plants under the dry water regime having less growth than those under the wet regime throughout the entire course of the experiment. This result is not surprising given that tall fescue is a C_3 species that cannot perform well during warm temperatures unless adequate water is supplied (Hannaway et al. 1999). The effects of the dry water regime were equally detrimental for both E+ and E- plants and across burn treatments. This was surprising, given that others have observed differences in growth response dependent on endophyte presence, especially under dry conditions (Arachevaleta et al. 1989; Elbersen & West 1996; Buck et al. 1997; Assuero et al. 2000; Bayat et al. 2009), although in some cases these effects have varied by host plant genotype (Elbersen & West 1996; Buck et al. 1997). It is possible that my 'dry' treatment was not dry enough to stimulate such endophyte effects, although it should be noted it was dry enough to depress tall fescue growth.

Endophtye effects on biomass were opposite those expected (E- > E+), and as stated previously, there were no significant interactions with water regime or burn treatment. The only time E+ plants had higher biomass than E- was for initial root weight. E+ fescue has been shown in a number of cases to have greater shoot (Arachevaleta et al. 1989; Clay 1990; Belesky & Fedders 1995; Hill et al. 1998; Assuero et al. 2006; Pecetti et al. 2008) and root (Belesky & Fedders 1995; Kelrick et al. 1990; Assuero et al. 2006) mass compared to E-. However, differences in the previously mentioned studies vary widely (e.g., E+ plants 4.4% (Pecetti et al. 2008) to 70% (Assuero et al. 2006) more biomass than E-), and there are a few studies in which no endophyte effect was observed. Endophyte presence did not affect leaf elongation, tiller density or

dry weight per tiller in studies conducted by Elbersen and West (1996) and Newman et al. (2003). It did result in earlier flowering in the Newman et al. (2003) study, but in my experiment, date of flowering was not significantly affected by endophyte presence either. Some might speculate that endophyte effects are better seen in field studies than in greenhouse studies, but in a climate change experiment in the field at the same research farm from which I collected tall fescue (and using tall fescue propagated from seed collected in the plots from which I collected plants), Brosi (2011) also observed relatively few endophyte effects on tall fescue tiller growth. Host plant genotype (Elbersen & West 1996; Buck et al. 1997; Assuero et al. 2000; Roberts et al. 2002; Timper et al. 2005; Pecetti et al. 2008) and fungal genotype (Assuero et al. 2000; Roberts et al. 2002; Timper et al. 2005; Belesky et al. 2008; Rudgers et al. 2010) have both been shown to influence the dynamics of symbiosis within the tall fescue-*N. coenophialum* system, and it may be that the combination used in my study simply doesn't exhibit the differences in growth seen in other cases.

Physiological benefits of symbiosis with *N. coenophialum* to host plants can vary depending on soil fertility (Arachevaleta et al. 1989; Cheplick et al. 1989; Malinowski et al. 1997), but the patterns across studies are not consistent. Cheplick et al. (1989) found higher biomass of E+ seedlings compared to E- at high nutrient levels and lower biomass for E+ at low nutrient levels, but Arachevaleta et al. (1989) and Malinowski et al. (1997) saw higher biomass for E+ at lower nutrient levels and no differences (Arachevaleta et al. 1989) or reduced biomass for E+ (Malinowki et al. 1997) at high nutrient levels. The plants used in the current study were grown in the relatively fertile (especially for P; see table 4.3) soil from which they originated. Malinowski et al. (1997) and Rahman and Saiga (2005) looked at tall fescue growth in response to different P levels, and my results are consistent with what both found in high P soils, E+ biomass was lower than E-. It may be that if I had performed this experiment in less fertile soil I would have seen a different outcome with regard to the potential endophyte effects on growth. Given the variability in growth responses in previous studies and this one, it

seems there is still much to be learned about the conditions under which fungal endophyte symbiosis should be considered mutualistic as opposed to parasitic (which seemed to be the case in my study) for this species.

The response of tall fescue to fire might be dependent on its life history (specifically life form and bud characteristics), which Pyke et al. (2010) used to characterize plant species' fire tolerance. With tall fescue being a cryptophyte (sensu Raunkiaer 1934), Pyke et al. (2010) predicted the growth response following fire to be neutral or positive if buds are insulated by soil, but negative if buds are closer to the surface and fire temperatures are hot enough. In a review of fire effects on invasive weeds, DiTomaso et al. (2006) list cool-season perennial grasses as a category that can be controlled with burning, and while they do not specifically address tall fescue, they do cite successful reductions in Kentucky bluegrass with mid-late spring burns. However, in my study, tiller length was greater for the burned pots compared to the control, but biomass (tiller and pot total) was suppressed in 2x compared to 1x or unburned control, so there was a slight reduction in material burned 2x at the end of the experiment (leaf sheaths were the same lengths but apparently not as thick). The rapid recovery in length following the second burn was suprising, and likely indicates that given more time prior to harvest (2x burned plants were harvested only 59 days after the second burn, but 1x burned plants were harvested 117 days after the first burn) the 2x burn pots may have regrown all, if not more than, the material lost to fire.

I did not observe a significant water*burn treatment effect on tall fescue growth. It may be that prescribed fire during a dry time (when tall fescue growth may already be suppressed) and during either the spring/early summer or autumn growing period would have a negative impact on tall fescue. My study did not impose this combination of conditions (watering regimes started the day of the first burn so soil moisture was the same at that time, and the second burn was during slow mid-summer growth), but these conditions may also pose a safety risk that would preclude burning, and/or

negatively impact desired plant species (in the case of autumn burning when native warm season grasses are present).

Tall fescue experiences two periods of growth during a single season with a period in the mid-summer of slow growth (Lacefield et al. 2003), and it is possible that the timing of fire might interact with the seasonal growth cycle of tall fescue to alter the plant's response. Based on growth rates prior to burns, this experiment imposed the first burn during the period of early summer growth, and tall fescue took longer to recover compared to when the second burn was applied, which occurred as the plants were entering their slower growth mid-summer period. Prescribed fires are most often conducted in February or March in Kentucky, which is when our first prescribed burn coincided with (during the initial spring growth period). Based on my data, a burn applied at this time appears to allow plenty of time for plants to recover aboveground material and they can do so in a relatively short period of time (~3 weeks in this greenhouse experiment). A burn during the mid-summer period (which is when the second burn in this experiment occurred) resulted in rapid recovery in length (2x burn plants had the same tiller length as 1x and unburned control within 2 weeks following the second fire). A summer prescribed burn applied to a field dominated by another C_3 grass, Texas wintergrass, resulted in 2x higher yield of that species compared to a winter (Feb/Mar) burn or no burn (Ansley & Castellano 2007). A burn during the autumn growing period would allow less time for recovery before the winter dormant period, and might be predicted to reduce tall fescue dominance better over the long-term than summer or spring burns, but Madison et al. (2001) found that fall burning did not reduce tall fescue cover. My results indicate that, as others have found, prescribed burning alone is not likely to significantly reduce tall fescue cover (Washburn et al. 2000; Madison et al. 2001; Barnes 2004), even if applied twice in a single growing season, under wet or dry conditions, and irrespective of endophyte status.

Conclusions

My data suggest that regardless of endophyte status, tall fescue being targeted for removal in grassland restorations will need to be treated with herbicide in addition to prescribed burns, which alone (even twice in one season) stimulated fescue growth in this experiment . Water stress negatively affects tall fescue growth, and did so equally for E+ and E- plants in this experiment. When I did observe significant effects of endophyte on growth of fescue plants, it was opposite that expected, with E- plants having greater tiller length and biomass compared to E+. These results add to the growing body of literature that shows differences in E+ and E- tall fescue plant response to stress may depend on a number of factors (i.e., soil fertility, tall fescue and fungal endophyte genotype interactions, climatic factors, etc.) and are not universal across its range in the Eastern U.S. My data suggest that prior knowledge of endophyte status of tall fescue pastures targeted for renovation to native grassland species is not necessary.

Tables

Table 3.1. P-values for effects of burn treatment (1x, 2x, unburned control), water regime (dry, wet), and endophyte infection status(E+, E-) and their interactions on biomass measurements and tiller number at the final harvest. NS indicates *p*-values >0.05.

	Total Length	Number New Tillers	Number Reprod. Tillers	Tiller Biomass	Root Biomass	Total Biomass
Burn Treatment	0.0066	NS	0.0015	<.0001	NS	0.0002
Water Regime	<.0001	<.0001	0.0235	<.0001	0.0001	<.0001
Endophyte	0.0256	NS	NS	0.0129	NS	NS
Trtmt*Water	NS	NS	NS	NS	NS	NS
Water*Endophyte	NS	NS	NS	NS	NS	NS
Trtmt*Endophyte	NS	NS	NS	NS	NS	NS
Trtmt*Water*Endophyte	NS	NS	NS	NS	NS	NS

Table 3.2. Mean measured growth response variables (± 1 S.E.) for tall fescue plants exposed to 1 prescribed burn (1x), 2 prescribed burns (2x), or no prescribed burn (control), averaged across watering regimes and endophyte status. Parameters shown are those for which burn treatment had a significant main effect (see Table 3.1 above). Letters represent LS Means differences for the main burn treatment effect.

	1x	2x	Control
Total Tiller Length (cm)	230.9±17.9 a	221.3±19.5 a	178.8±12.4 b
Reprod Tillers (#)	0.25±0.1 b	0.23±0.1 b	0.75±0.2 a
Tiller Biomass (g)	2.60±0.16 a	1.89±0.15 c	2.23±0.13 b
Total Biomass (g)	5.60±0.31 a	4.43±0.23 b	5.28±0.30 a

Table 3.3. Mean measured at the final harvest growth response variables (± 1 S.E.) for tall fescue plants exposed to Wet and Dry water regimes and averaged across burn treatments and endophyte status. Parameters shown are those for which watering regime had a significant main effect (see Table 3.1 above). Letters represent LS Means differences for the main water regime effect.

	Wet	**Dry**
Total Tiller Length (cm)	264.9±10.8 a	155.2±10.2 b
Number New Tillers (#)	11.0±0.9 a	4.0±0.8 b
Reprod. Tillers (#)	0.6±0.1 a	0.3±0.1 b
Tiller Biomass (g)	2.78±0.08 a	1.72±0.10 b
Root Biomass (g)	1.84±0.08 a	1.36±0.08 b
Total Biomass (g)	6.10±0.17 a	4.16±0.18 b

Table 3.4. Mean measured growth response variables (± 1 S.E.) for E+ and E- tall fescue plants averaged across watering regimes and burn treatments. Parameters shown are those for which endophyte presence had a significant main effect (see Table 3.1 above). Letters represent LS Means differences for the main endophyte effect.

	E+	**E-**
Total Length (cm)	193.9±14.8 b	225.3±14.8 a
Tiller Biomass (g)	2.12±0.13 b	2.37±0.13 a

Figures

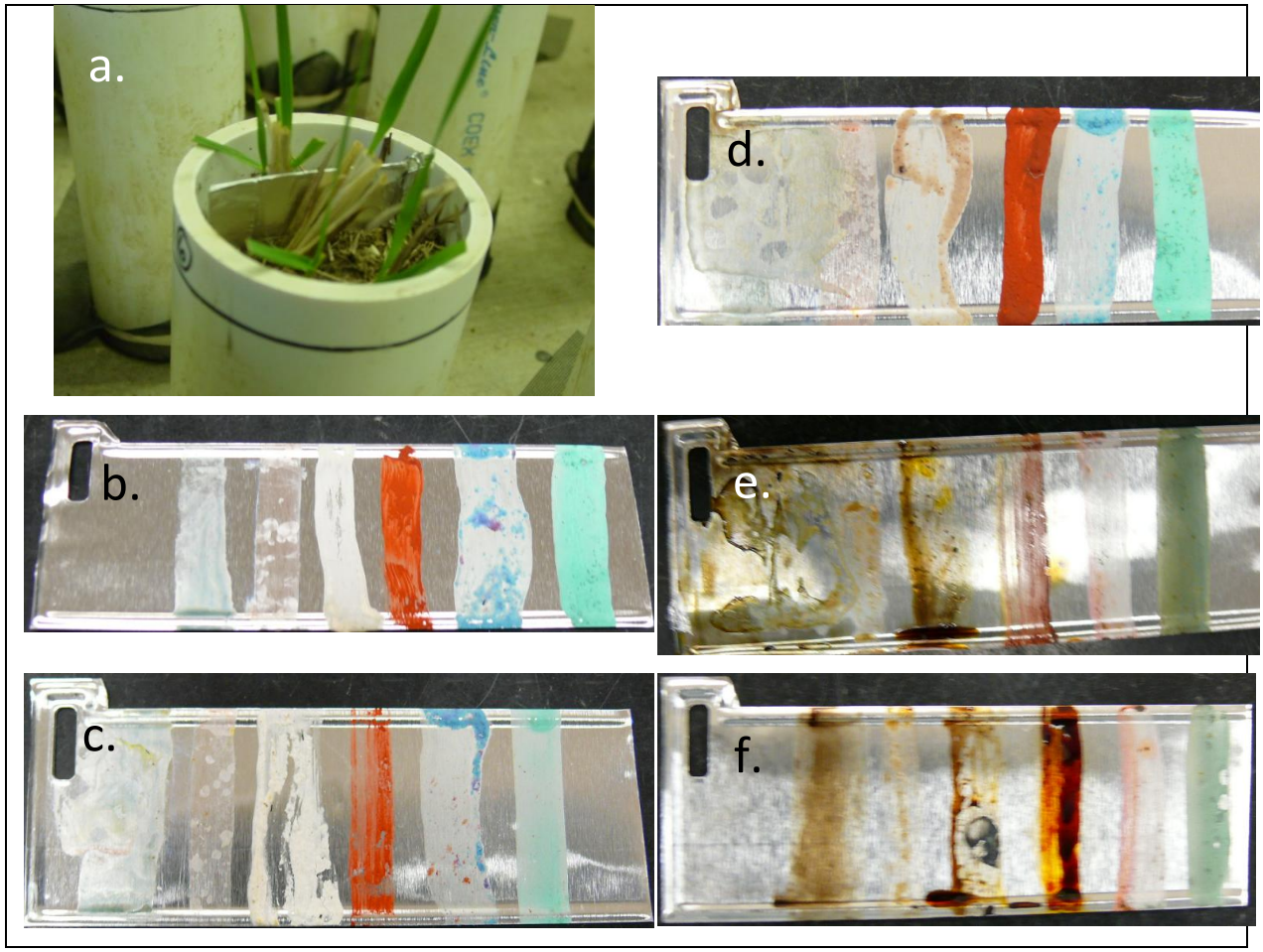


Figure 3.1. Tags with heat-sensitive paints, situated on the soil surface of a "pot" prior to warm season grass litter being put into place and the prescribed burn treatment performed (a), and with no paints burned (b; <79˚C), the first paint burned (c; 79-162˚C), the second paint burned (d; 163-245˚C), the third paint burned (e; 246-315˚C), and the fourth paint burned (f; 316-398˚C). Each pot had a single metal tag wrapped in aluminum foil to evaluate the temperature obtained during burning.

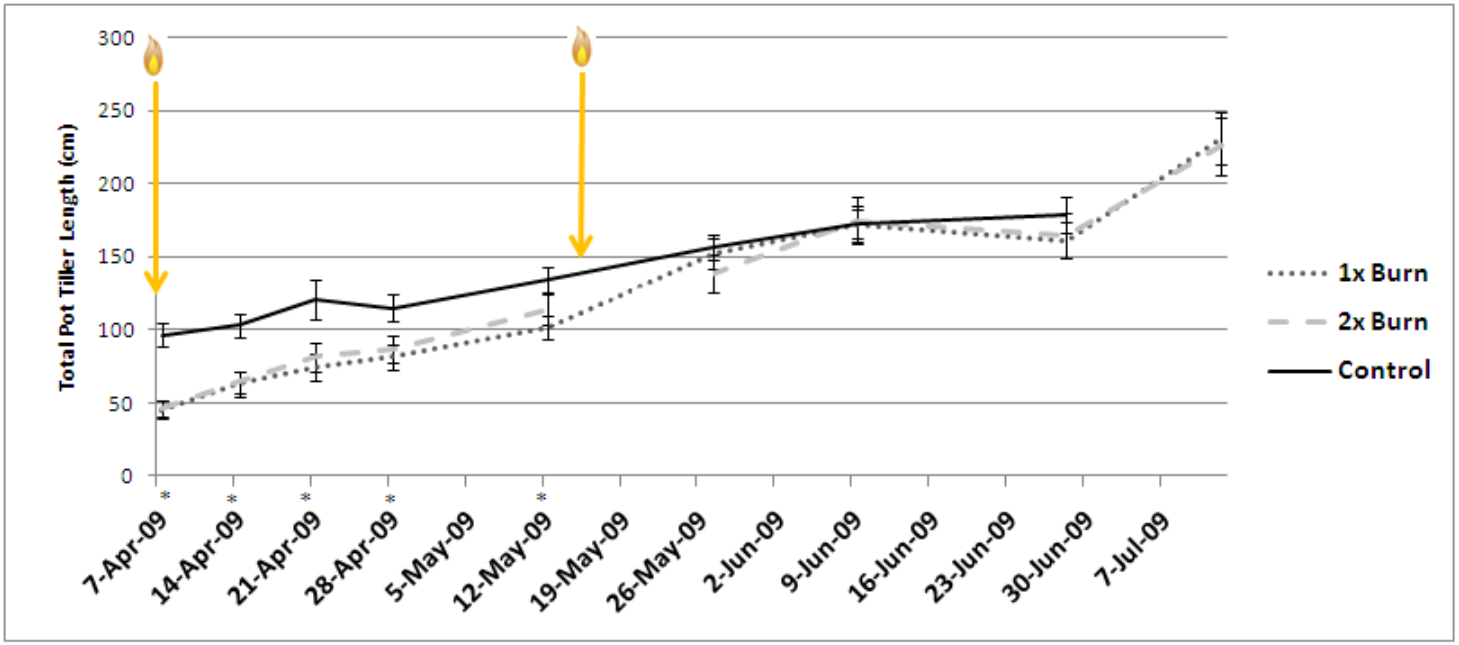


Figure 3.2. Average total pot tiller length (± 1 S.E.) across the duration of the experiment, as measured at each of nine measurement intervals (measurements for all treatments were made within a two-day window for each interval). Asterisks indicate dates for which there was a significant difference between treatment means. Flame symbols indicate when the two prescribed burn treatments were applied to either both the 1x and 2x treatments for the first burn (25 March), or the 2x treatment only for the second burn (12 May).

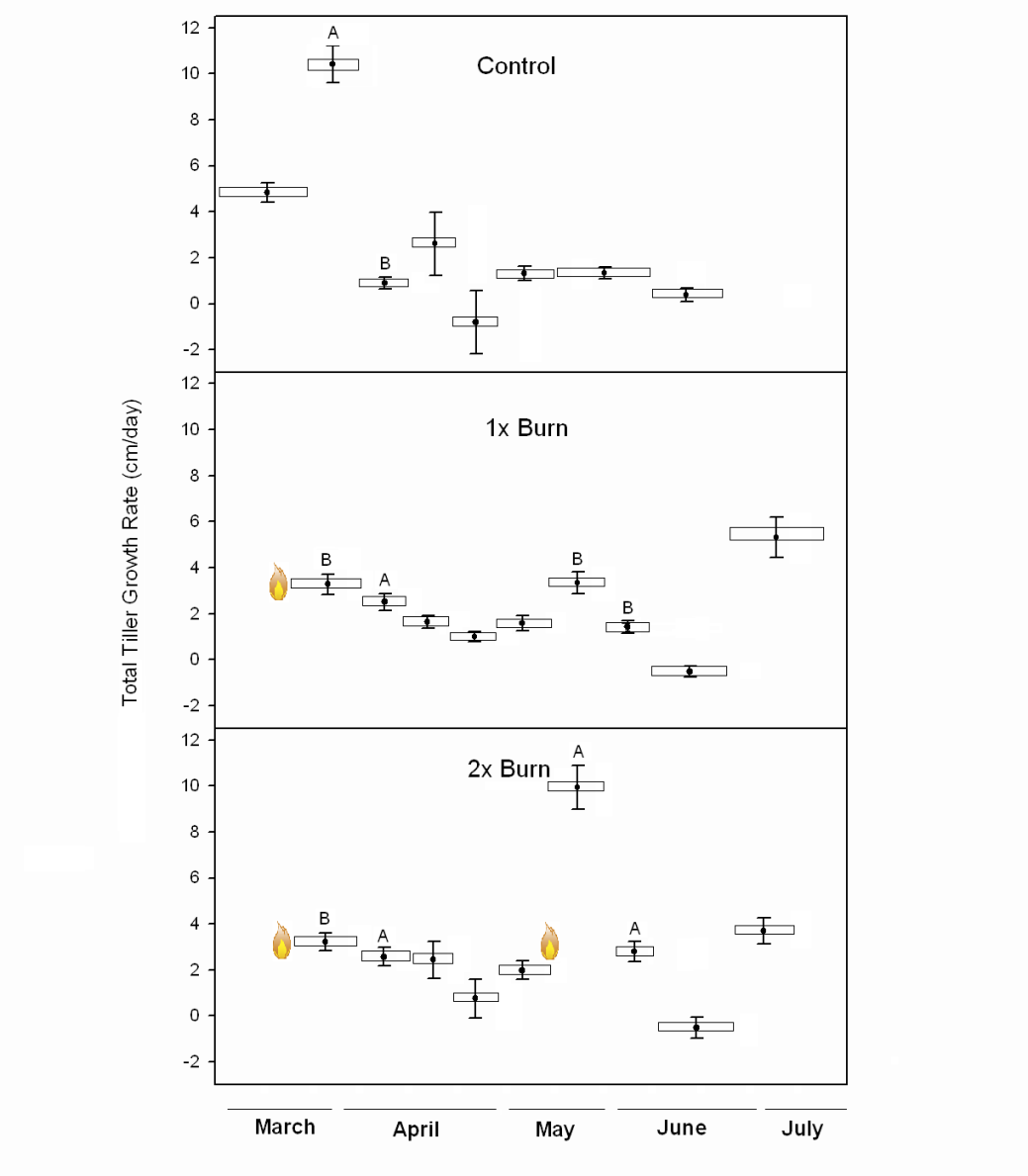


Figure 3.3. Mean total tiller growth rate (cm/day) (±1 S.E.) for tall fescue pots under different burn treatments (unburned control, 1x, 2x). Timeline of the experiment is represented on the x-axis, and the width of bars accompanying each point reflects the time period for which the growth rate was calculated. Flame symbols indicate when prescribed burn treatments were applied. Letters above error bars indicate significant differences between burn treatments within a given measurement interval.

Chapter Four

Does endophyte infection of tall fescue pasture alter potential mycorrhizal colonization of native species commonly planted in grassland restorations?

Introduction

Given the widespread distribution and abundance of tall fescue in the eastern United States, the issues regarding tall fescue and forage quality (e.g. Rhodes et al. 1991, Ball et al. 1993, Bacon 1995), its negative effects on wildlife habitat (Barnes et al. 1995, Madison et al. 2001), and its potential to invade into surrounding areas (Spyreas et al. 2001, Rudgers et al. 2005), tall fescue pastures are being targeted for grassland restoration throughout its range. The goals of land managers are to eliminate tall fescue and increase native species cover. Some practitioners refer to areas that are managed without the intentional introduction of native seed as "restorations," and those in which native seed is introduced as "renovations."

A common approach used in tall fescue pasture renovations is to apply a broad-spectrum herbicide to kill off all plants, followed by the use of a no-till drill to seed desirable native species (Harper et al. 2004; Barnes 2004). This approach has been shown to be even more successful if the herbicide application is preceded by a dormant-season prescribed burn (Washburn et al. 2002). With this approach, seeds are planted directly into soil previously dominated by tall fescue. Limited evidence suggests endophyte-infected (E+) fescue may suppress mycorrhizal fungi in the soil (Chu-Chou et al. 1992; Antunes et al. 2008), which could affect successful germination and/or establishment of planted native seeds.

Arbuscular mycorrhizal fungi (AMF) include fungi from the phylum Glomeromycota (Schüssler et al. 2001) and are known to form associations with root systems of a wide variety of plant species (Smith & Read 1997). This group includes those formerly referred to as "vesicular-arbuscular mycorrhizae" or VAM. These fungi

are thought to enhance growth (McGonigle 1988) of many plant species, and are hypothesized to have formed associations early in the evolution of land plants (Brundrett 2002). Levels of plant association with AMF vary, with some species considered non-dependent (not usually in association with AMF), some considered facultative (may or may not have association with AMF), and some considered obligate or dependent (almost always found in association with AMF). In an exhaustive review of 6,507 angiosperm species (Trappe 1987), 67% had AMF associations. In a more recent survey, only 53 of 336 Angiosperm families were found to be non-mycorrhizal (Brundrett 2009). The genus *Glomus* dominates this soil group (e.g. Dhillion 1992; Eom et al. 1999; Gai et al. 2009; Huag et al. 2010), and has been clearly linked with transformation of organic phosphate (Koide & Kabir 2000). AMF from the family Gigasporaceae have also been linked with P uptake by host plants, while those in the family Glomeraceae (to which the genus *Glomus* belongs) have been linked to protection against pathogens (Maherali & Klironomos 2007).

The degree to which association with AMF affects growth of a host plant varies by species, with some exhibiting no difference or even negative growth when associated with AMF, while many others exhibit clear positive growth responses (McGonigle 1988). Symbiosis with AMF has been described as a continuum from parasitic to mutualistic, with an individual plant's response depending on its own species identity, the individual fungal species, and the community of fungi present (Klironomos 2003). The degree of association can also be impacted by abiotic factors in the environment, especially availability of P and N, which the fungus transfers to the plant (Pringle et al. 2009). When the response of a plant to microbial symbionts is studied, sterilization or pasteurization of soils, which removes the microbes of interest, is often used as a mechanism through which comparisons can be made.

Abundance of AMF in soil has been shown to differ greatly depending on soil quality and plant community composition. Piotrowski et al. (2008) looked at a 70 year chronosequence in Montana, USA, and found AMF abundance peaked during the first

13 years of succession, after which the fungal community shifted to ectomyccorhizal fungi. Velazquez et al. (2010) compared diversity of AMF from five vegetation types, and found higher diversity in grassland and palm forest compared to gallery forest, marsh, or scrubland.

Soil fertility has been linked with overall soil microbial abundance and community structure, with microbial abundance often inverse to fertility. Soils receiving regular nitrogen addition have lower microbial abundance than low fertility soils, and highly fertile soils are dominated by bacteria, while lower fertility soils are often dominated by fungi (Bardgett et al. 1997; Brodie et al. 2002; Grayston et al. 2004; Bradley et al. 2006). AMF have been clearly demonstrated as important in the acquisition of P for host plants (e.g. Lekberg et al. 2010), and the degree of association has been linked with P availability. In soils where P is more available, fewer AMF associations generally occur (Hetrick et al. 1990; Eom et al. 1999), and in soils where P is more limited, more AMF associations generally occur (e.g. Hetrick et al. 1990; Anderson et al. 1994; Anderson 2008). Plant-AMF symbiosis has also been linked to acquisition of nutrients from soil organic matter (reviewed by Talbot et al. 2008). Anderson et al. (1984) looked at plant cover, species richness, and AMF spore abundance across a soil moisture and nutrient gradient, and found significant positive correlations of plant cover, species richness, and AMF spore abundance with each other, as well as organic matter. They also observed that association with AMF for three plant species was more common in the drier, nutrient poor soils in their gradient, than the wetter, higher nutrient soils, where they routinely did not find associations.

The role of AMF in grassland ecosystems, which are often P limited, is typically considered quite important, with colonization occurring in many common grasses and forbs (Anderson et al. 1984; Miller 1987; Wilson & Harnett 1998). Grasses are generally considered less mycotropic than forbs due to their highly fibrous and branched root systems (Baylis 1974), but when examined for mycorrhizal colonization, they frequently contain the symbionts. Studies have focused on a number of dominant North American

C_4 grasses, including *Andropogon gerardii* (Dhillion 1992; Brejda et al. 1993; Anderson et al. 1994; Hartnett et al. 1994; Wilson & Hartnett 1997, 1998; Bingham & Biondini 2009), *Schizachyrium scoparium* (Dhillion 1992; Meredith & Anderson 1992; Dhillion & Anderson 1993; Anderson et al. 1994; Hartnett & Wilson 1999; Burrows & Pfleger 2002; Anderson 2008; Bingham & Biondini 2009), *Panicum virgatum* (Brejda et al. 1993; Hartnett et al. 1994; Bingham & Biondini 2009), and *Sorghastrum nutans* (Wilson & Hartnett 1997; Bingham & Biondini 2009). When C_4 grasses have been compared to C_3 species in terms of AMF colonization (Hetrick et al. 1990), C_4s exhibited far greater positive responses to and reliance on AMF, to the extent that under low P conditions, non-mycorrhizal C_4 plants did not grow. C_3 grasses, on the other hand, more frequently did not respond to AMF or P amendment (Hetrick et al. 1990; Wilson & Hartnett 1998). Responses of forbs to AMF colonization are more varied, with some exhibiting strong positive responses, and others negative growth responses (Wilson & Hartnett 1998; Wilson et al. 2001).

A handful of studies have looked at interactions between tall fescue, its aboveground fungal endophyte (*Neotyphodium coenphialum)* and belowground soil biota, including AMF. Evidence suggests E+ tall fescue plants exhibit less association with AMF than endophyte-free (E-) plants (Chu-Chou et al. 1992; Mack & Rudgers 2008; but see Guo et al. 1992), and the abundance of mycorrhizal spores beneath E+ fescue was lower than beneath E- (Chu-Chou et al. 1992). In one study, tall fescue showed a significant positive response to mycorrhizal colonization (Wilson & Hartnett 1998), but the endophyte status of the plants used in this study was unknown. When fescue extracts were applied to AMF spores in the lab, germination of spores decreased by 10%, although there was no difference between E+ or E- extracts (Antunes et al. 2008). When *Bromus* plants were grown in a greenhouse and watered through E+ and E- fescue thatch, colonization of *Bromus* roots by AMF was reduced for plants with E+ compared to E- thatch (Antunes et al. 2008). Growth of three out of nine tree species grown in soil conditioned by E+ tall fescue was reduced compared to those grown in

soil conditioned by E- tall fescue, and three other plant species showed a non-significant trend for reduced growth in E+ soil (Rudgers & Orr 2009). Rudgers and Orr (2009) attribute this reduced growth response to an alteration of soil biota occurring as a result of endophyte presence. A recent study has shown that soils supporting E+ tall fescue ('Jesup' cultivar) had 26% less AMF lipid biomarker than E- soils (Buyer et al. 2011).

It is possible that the presence of the aboveground fungal symbiont directly affects AMF abundance in soils dominated by tall fescue; however, the photosynthetic mode of tall fescue and the fact that tall fescue often dominates the plant community in stands where it occurs may also influence the abundance of AMF in the belowground soil community. Tall fescue is a C_3 grass, which are generally considered less mycotrophic than C_4 species (Hetrick et al. 1990; Wilson & Hartnett 1998; Hoeksema et al. 2010). In areas dominated by plant species that are not reliant on microbial symbionts, the abundance of the symbionts may decrease over time, or conversely, when plants that are dependent on them increase in dominance, so too do the symbionts. This has been observed in the case of shifting fungal communities in forest succession (from AMF to ectomycorrhizal fungi; Piotrowski et al. 2008) and in the increased abundance of rhizobia (N-fixing bacteria) following the conversion of forest to pasture (Nüsslein and Tiedje 1999). Endophyte-infected tall fescue often serves as the dominant vegetation type where it occurs and supports lower plant diversity than its endophyte-free counterpart (Clay & Holah 1999). Plant diversity has been linked with abundance and diversity of AMF (van der Heijden et al. 1998; Mummey & Rillig 2006; Vogelsang et al. 2006), with soils occupied by less diverse aboveground vegetation having lower diversity and abundance of AMF. Together, these results suggest that areas dominated by E+ tall fescue may lack beneficial soil microbial symbionts (AMF and rhizobia) important for native grassland species, which could impact establishment and growth of those species when planted by seed into soil previously occupied by tall fescue during fescue renovation efforts.

Interactions between aboveground vegetation (and any microbial symbionts it contains), belowground vegetation, soil characteristics, and the soil microbial community are complex, and our understanding of these interactions as a whole is quite limited. The tall fescue-*Neotyphodium* symbiosis, which is commonly present in aboveground vegetation of pastures targeted for restoration of native species that form symbioses belowground with AMF and rhizobia, offers a unique system in which to explore these interactions. Comparisons of belowground symbiosis in seedlings of native grassland species, when planted into soils with different aboveground vegetation components, soil fertility, and land use history, can shed light on some of the interactions within this system, and may be useful information for land managers wishing to restore native grassland species in areas that are currently tall fescue pasture.

I designed an experiment to explore this system, by planting seeds of four native grassland species into soil from three different sites (two in Kentucky and one in Indiana), each containing three different stand types (E+ and E- tall fescue and forest), and I employed a sterilization treatment in an attempt to identify the role of the soil microbial community on plant production. As the three sites occurred in different regions of the central United States and were managed differently, I first quantified differences in basic soil parameters across sites and stand types. Then, I tested for differences in biomass production and root mycorrhizal colonization of the native seedlings grown in those soils. Plant species were chosen from the ones most commonly used in grassland restorations, and included two native C_4 grasses- *Panicum virgatum* L. (switchgrass), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem), a forb- *Echinacea purpurea* (L.) Moench (purple coneflower), and a legume- *Chamaecrista fasciculata* (Michx.) Greene (partridge pea). I developed general hypotheses for my research objectives based on patterns/trends from previous research, as well as known differences in vegetation and management between the sites.

I hypothesized that the presence of belowground microbial symbionts would be beneficial to the growth of seedlings overall, which would be evidenced by greater

biomass and root mycorrhizal colonization for seedlings in non-sterilized soil compared to sterilized. I hypothesized that root mycorrhizal colonization would be lowest in the high fertility (especially high P) soils, and highest in the low fertility soils. I expected to see differences in mycorrhizal colonization between stand types reflective of AMF abundance in the soil, with forested soils being the lowest, and E+ being lower than E-. Finally, I predicted there would be species-specific differences in mycorrhizal colonization reflecting differences in association and dependence on AMF.

Methods

Soil from three sites, each containing three vegetation stand types (E+ tall fescue, E- tall fescue, and a "fescue-naïve" or forested state labeled "FO"), were utilized in a split plot experimental design to evaluate the effects of tall fescue and fungal endophyte presence on biomass and mycorrhizal colonization of planted native species and the variability of these effects across sites. Four replicate soil samples were collected in each stand type at each location. Half of this material was steam sterilized. All fescue was the variety KY-31, and the endophyte present was the common toxic *Neotyphodium coenophialum*. Four native species: *Chamaecrista fasciculata* (Michx.) Greene (partridge pea), *Echinacea purpurea* (L.) Moench (purple coneflower), *Panicum virgatum* L. (switchgrass), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem) were planted from seed as individual plants in the different soil types. Hereafter the plant species will be referred to by their respective genus only. See figure 4.1 for a schematic of the experimental design.

Site Descriptions

The Indiana site (IN) is near Bloomington, Indiana (39.219167˚N -86.541389˚W), and was established in Fall 2000 for long-term research on the ecological effects of E+ and E- tall fescue. These plots were planted in a former agricultural field, and received no further management (ie. fertilization, mowing, etc.) (Rudgers & Clay 2008). Vegetation has been described between the eight (30x30m) E+ and the eight E- plots,

with the E- having higher plant species richness and more non-fescue aboveground biomass than the E+ plots (Rudgers & Clay 2008). Forested stand samples were collected along a stream that ran parallel to the edge of research plots, approximately 35 meters away (Fig. 4.2a). The vegetation for all forest stand samples was predominantly boxelder overstory, with sparse groundlayer vegetation.

Crooked Creek State Nature Preserve (CC) is in Lewis County, Kentucky (38.646508˚N -83.577198˚W), and was the location for the study presented in chapter two of this dissertation. The origin of tall fescue and exact planting date are not known for either of the stands at Crooked Creek, but according to interviews of residents at the time of KSNPC purchase (1999), it was planted in the 1950's (J. Bender, KSNPC, personal comm.). Soil from beneath E- fescue was collected from the sub-unit of the "Fescue" unit that had significantly higher tall fescue cover than all others in that study, sub-unit C (see chapter two). The E+ stand was within the preserve boundaries but in a management unit not discussed in chapter two (the "Hayfield" unit, see Fig. 4.2b). It was also formerly used as a pasture but since 1999 has been managed as part of the state nature preserve, receiving periodic prescribed burns and herbicide applications (less frequently than the "Fescue" unit). Neither of the fescue stands get mowed or receive applications of fertilizer. The forested stand was a patch nearby the E- stand (approx. 255 meters apart, see Fig. 4.2b). The overstory varied slightly between individual sample points, but was predominantly oaks (scarlet, post, white) with some sugar maple, white ash, and beech. Midstory species included serviceberry, dogwood, rusty blackhaw, and Japanese honeysuckle.

The third site, Spindletop (ST), is the same location where tall fescue was removed for the experiment presented in chapter three of this dissertation (39.219167˚N -86.541389˚W). The same research plots were used (planted in 2001), with sample locations in each half of the four strip plots (two E+ and two E-). These plots are managed on a regular basis as tall fescue seed plots, which includes application of 50lbs/acre N every Fall (Oct or Nov), mowing to 6-in after early and late summer seed

harvests (July, Sept), and occasional use of 2,4-D herbicide for broadleaf weed control. The forest stand was a patch (approximately 1-km away, Fig. 4.2c) with hackberry overstory, bush honeysuckle and hawthorn midstory, and wintercreeper groundlayer (wintercreeper was cleared to collect all soil samples).

Soil Collection, Treatments & Measurements

Soil was collected from these three sites during June 2010. Within each stand type at each site, four sample areas (5x5m each, at least 12m apart) were identified, and replicate soil samples were taken from beneath two 0.25x0.25m quadrats within each sample area. Quadrat locations were chosen to maximize the amount of tall fescue directly above where the soil was collected. Tall fescue cover was visually estimated and 6 fescue tillers removed from each quadrat (Table 4.1). Tillers were composited together for each replicate quadrat, and were tested for presence of *N. coenophialum* using Phytoscreen Immunoblot kits (Agrinostics, Watkinsville, GA). Endophyte infection frequency was determined by dividing the number of tillers testing positive for the presence of *N. coenophialum* by the total number of tillers tested per sample area (n=12) (Table 4.1). Fescue sod or leaf litter (in the forested stands) was removed to expose soil directly beneath it, which was sampled to approximately 10-cm in depth for the whole quadrat area using a straight-edged shovel and trowel (~2.5 liters). Tools were wiped clean between sampling to minimize contamination.

All soils and fescue tillers were kept cool during transport and refrigerated once in the lab. Soils from the two quadrats for each replicate sample area were sieved through a 4.75mm sieve and combined to create one homogenized sample (~ 5 liters). A 5-g subsample of each was taken and stored at -80˚C for phospholipid fatty acid (PLFA) analysis. An additional 10-g subsample ("pre-sterile") was collected for analysis of pH, macronutrients, micronutrients, and total C & N by University of Kentucky Division of Regulatory Services. The remaining composite soil samples were then split in half, with one half spread to 4cm depth in a metal tray and steam sterilized at 105˚C and 20psi for

100 minutes. 3M Comply SteriGage tape was used to ensure that sterilizing conditions were achieved. Sterilized soils were double bagged and placed in cold storage immediately after sterilization.

PLFA is used to detect differences in abundance of broad microbial taxonomic groups, which can be identified based on their tendency to produce specific lipid biomarkers (White 1995). We did not perform this procedure on all soils, but chose two subsets of samples to explore patterns in total biomass of microbes (Total PLFA) and relative abundance of arbuscular mycorrhizal fungi, characterized by the lipid marker 16:1ω5 (van Diepen et al. 2010). The first set of subsamples analyzed with PLFA was aimed at detecting differences in microbial composition prior to and following steam sterilization, and included the composite sample from each of the four sample areas for E- soil at Spindletop only (non-sterilized and sterilized, 8 samples total). The second set of subsamples analyzed with PLFA was aimed at detecting differences in microbial composition between different stand types across sites, and included one composite sample made across the reps (sample areas) for each site*stand combination (non-sterilized only, 9 samples total). Methods follow that of D'Angelo et al. (2005) and Weand et al. (2010).

Greenhouse

Soil samples (~2.5 liters non-sterilized and 2 liters sterilized for each site*stand*replicate sample, n=36) were taken to the research greenhouses at the Kentucky State University Research Farm (Franklin County, Kentucky 38.116065˚N -84.890506˚W). A 10-g subsample of each was collected immediately before planting to be analyzed for the same soil properties as listed for the "pre-sterile" subsamples. In addition, textural analysis was done on the non-sterilized subsamples. The remaining sample (2.0 or 2.5 liters minus 10-g subsample) was mixed with an equal volume of potting media (Lambert Growing Mix) to achieve a 50/50 soil:growing media mix. This mix was then divided among 20 standard 164ml cone-tainers (Ray Leach, Inc.) (see

Figure 4.1). All containers and tools used during the mixing process were sprayed with water from a hose at high pressure until no soil was visible between uses to minimize contamination between different soil samples. After all soil samples had been mixed and divided into the appropriately marked cone-tainers, native seeds (provided by Roundstone Seed Company, Upton, KY) were planted. Seeds of *Chamaecrista* were pre-soaked in de-ionized water for 4 hrs to aid in germination, as its seeds are under physical dormancy (Baskin & Baskin 1998), which can be broken by imbibing seeds. For *Chamaecrista*, *Echinacea,* and *Schizachyrium*, three seeds were placed in each cone-tainer. For *Panicum*, whose seeds were small and hard (and thus difficult to handle individually), two tweezers full (approximately 7-15 seeds) were placed in each cone-tainer. Lambert growing mix was used to cover all seeds. The total number of cone-tainers planted per species was 3 sites x 3 stand types x 4 replicate samples x 2 sterilization statuses x 5 replicate plants=360. Cone-tainers were arranged in five blocks along a single greenhouse bench, and were watered daily. HOBO temperature sensors were used to monitor temperature in the greenhouse, with a reading taken every 30 minutes. These readings showed the mean temp for the duration of the experiment was 26˚C. Seedlings were under ambient light conditions throughout the experiment.

Two weeks after planting, seedlings were thinned to the largest single individual in each cone-tainer. Cone-tainers without a single seedling (256 *Chamaecrista*, 260 *Schizachyrium*, 45 *Panicum*, and 16 *Echinacea*) were replanted, with either three seeds for *Chamaecrista* (these were soaked as previously described prior to planting) and *Echinacea*, or two tweezers full of seeds for the grasses. These were covered once again with only the growing mix. Thinning and weeding (although few volunteers appeared) was done as needed. Seedlings were grown for two months total, at which time 301 *Chamaecrista*, 350 *Echinacea*, 349 *Panicum*, and 288 *Schizachyrium* cone-tainers contained live plants. Harvest was done in order of planting date to ensure all seedlings had the same growth period; replanted seedlings were harvested two weeks after all that had emerged from original planting.

Plant Harvest & Mycorrhizal Assessment

At harvest, roots of each plant were carefully washed under running water to remove all soil. Flowers were noted when present for *Chamaecrista* (not present in any other species). Aboveground and belowground plant material was separated and placed in brown paper bags or coin envelopes. All material was dried for 48 hrs at 60˚C, and biomass was measured immediately after removal from the oven. Biomass samples were then stored at room temperature.

Subsamples of roots from each plant were removed for mycorrhizal colonization assessment. I chose to omit *Chamaecrista* from this part of the study, given 1) that N-fixing species do not respond to AMF as much as other plant groups (Hoeksema et al. 2010), 2) the lack of response to AMF presence in this same species that was observed by Holah & Alexander (1999), and 3) the large number of samples and the time involved in mycorrhizal assessment, doing assessment for *Echinacea* and the two grasses. From each plant's root system, three 1x1cm subsamples were removed using scissors- one at the top, one in the middle, and one at the bottom of the root sample. For root systems too small to sample this way (not possible to remove 1x1cm square from the top, middle, and bottom), all material was cut into 1-cm length sections and was used as the sample. These subsamples were placed in biopsy cassettes with 0.9mm square openings (Ted Pella, Inc.). Cassettes were then cleared for 20 minutes in hot 10% KOH. After rinsing well, 2% HCl was added and remained covering the cassettes for 30 minutes. HCl was drained and hot 0.05% trypan blue was added to cover cassettes, soaking for 40 minutes. Cassettes were then removed and were stored for at least 48 hours in the refrigerator suspended in 1:1 glycerol:H_2O.

Each cassette was emptied into a petri dish, and 10 1-cm root sections were placed on a standard microscope slide for mounting. PVLG glue (INVAM Undated) was used to place cover slips on slides which were then all dried for 48hrs at 55˚C. Slides were scored for presence of arbuscules, vesicles, hyphae, or absence using the

magnified intersections method (McGonigle et al. 1990), slightly modified. We used magnification at 400x and 30 discrete "views" per slide (Figure 4.3), collected by moving the microscope stage along six lines, each intersecting five root pieces (15 views for the left column of five roots, and 15 for the right column of five roots). Each view was scored (based on where the cross-hair in the eyepiece transversed the root) as either: arbuscule, vesicle, hyphae, or none. In cases where an arbuscule and hyphae, or vesicle and hyphae were present together (vesicle and arbuscule never appeared together), it was scored as only arbuscule or vesicle. Percent AMF colonization or mycorrhizal colonization (MC) was calculated as: (arbuscules+vesicles+hyphae)/30.

Statistical Analyses

Biomass, mycorrhizal colonization, vesicle abundance, and arbuscule abundance were analyzed together for all species using a generalized linear model (Proc GLM) in SAS 9.2 (Cary, NC) to test for significance of site, species, stand type, sterilization, and all interactions. All data were square root transformed due to abnormal distribution. Replicate sample within stand was used as an error term for stand, site, and site*stand effects, with the residual error used for the remaining fixed effects (sterilization, species, stand*sterilization, site*sterilization, species*sterilization, stand*species, site*species, site*stand*species, stand*species*sterilization, site*species*sterilization, site*stand*sterilization, and site*stand*sterilization*species). I also ran the same generalized linear model (minus the species effects) for mycorrhizal colonization and biomass for each species separately (error terms same as listed for the main model). For soil parameters (P, Ca, K, % N, % C), Proc GLM was used to test for the effects of site, stand, sterilization, and their interactions, with residual error only (no reps within each replicate sample). The following transformations were made to improve normality of distributions: P, square root; TotalN, TotalC, log; Ca, K, none. Least Square Means (LSMeans) procedure was used to determine significant differences between means for all models, and differences were considered significant at α=0.05. For selected PLFA data (total PLFA and the relative abundance of 16:1ω5), Proc GLM and LSMeans were

used to test for significance of main effects (Site, Stand, Sterilization) but no interactions, due to lack of replicates for this analysis. Means and standard error for all data were calculated using the Means and Standard Deviation procedure in JMP 9.0 (SAS Institute, Cary, NC). Linear regressions of MC and plant biomass were run in JMP 9.0.

Results

Differences in Soil Parameters & Effect of Steam Sterilization

Site significantly affected all soil parameters (Table 4.2). Crooked Creek had significantly higher %C and K, and significantly lower P compared to soils from the other two sites (Table 4.3). Spindletop soils had the highest P, with approx 4.5x the amount in Indiana soils, and 14x the amount in Crooked Creek soils. Percent C was significantly lower for Indiana soils than the other two sites (Table 4.3). Stand had a significant effect for all soil parameters except K (Table 4.2). Carbon concentrations were significantly different across all three stand types (forest > E- > E+)(Table 4.3). While the site*stand interaction was not significant for %C, it was apparent that the differences between forest and fescue soils were consistent across all three sites, but the E- vs. E+ difference was primarily driven by differences observed between these two stand types at Crooked Creek (Table 4.3). There were no significant effects of site or stand on Total PLFA biomass or relative abundance of the AMF lipid biomarker 16:1ω5 (data not shown).

The Site*Stand interaction was significant for %N, P, and Ca (Table 4.2). For %N, E+ and E- soils had similar soil N levels at Indiana and Spindletop, lower than forest soils; however, at Crooked Creek, E- soils had higher %N than E+, more comparable to the forested soil at this site (Table 4.3). Indiana and Spindletop soils also showed the same pattern for P, with higher levels in the forest stand samples compared to the fescues and both E+ and E- fescue stands being similar; however, the soils from Crooked Creek were statistically similar with regard to P levels (Table 4.3). Calcium concentrations in forest soils at Indiana were ~2x that measured in either the E+ or E-

fescue stands; however, at Crooked Creek, the E- tall fescue soil had more Ca than the other two stand types, and at Spindletop, differences in Ca concentrations across stand types were more subtle but tended to be forest > E- > E+.

Sterilization significantly increased P (102.74 ±14.65 mg/kg vs. 71.27±12.43 mg/kg for sterilized and non-sterilized soil, respectively) equally across all stands and sites (data not shown), but affected no other soil nutrient parameters. For the set of samples chosen (E- from Spindletop), sterilization significantly decreased both total PLFA (p=0.043; 70.39±7.92 nmol PLFA g^{-1} dry soil vs. 95.21±8.87nmol PLFA g^{-1} dry soil, sterilized vs. non-sterilized, respectively) and the relative abundance of 16:1ω5 (p=0.0007; 3.03±0.19% sterilized vs. 5.14±0.27% non-sterilized).

Biomass

There was a significant main effect of site on biomass of all species when analyzed together (Table 4.4), with Crooked Creek having, on average, lower biomass than the other two sites (Fig. 4.4a). Stand did not significantly affect all species combined, but did affect *Chamaecrista* and *Panicum* when analyzed independently (Table 4.5). The site*stand interaction was significant for all species combined (Table 4.4) and for all species alone except for *Schizachyrium* (Table 4.5). For all species combined, seedling biomass did not differ significantly between stands at Indiana; however, for Crooked Creek, seedlings had the highest biomass in the E+ soils, while for Spindletop, biomass was significantly different between all stands, and was lowest in E+ soils and highest in soil from the forest stand (Fig. 4.4a). Although when run by site for *Chamaecrista,* the stand effect was not significant (Fig. 4.5a-c), the full model for this species (Table 4.5) suggests that it was, with FO biomass being lower than E+ or E-. On an individual species basis in non-sterile soils only, *Chamaecrista* had similar biomass trends across stands at Indiana and Spindletop (E- > E+ > FO), but at Crooked Creek, E+ soils supported more seedling biomass than either E- or FO (Fig. 4.5a-c). For *Echinacea*, seedling biomass did not differ dramatically between stand types at Indiana

or Crooked Creek, but at Spindletop FO > E+ (non-sterile soils only, Fig. 4.6a-c). *Panicum* seedlings had higher biomass in forested stands compared to fescue soils for all three sites, being significant for Crooked Creek and Spindletop (non-sterile soils only, Fig. 4.7a-c). The main site effect for *Schizachyrium* reflected lower biomass in soils from Crooked Creek compared to the other two sites. Indiana soils supported the highest biomass for *Schizachyrium*, regardless of the stand type, as neither stand type nor the stand*site interaction was significant for this species (non-sterile soils only, Fig. 4.8a-c).

Biomass was significantly affected by sterilization (Table 4.4), but the effect varied among species. For the three non-N fixing species, biomass was significantly higher in sterilized soils, but for *Chamaecrista* it was significantly lower in sterilized soil compared to non-sterilized (Fig. 4.9a). The stand*sterilization interaction was significant for biomass of all species combined (Table 4.4), and for *Chamaecrista* and *Echinacea* alone (Table 4.5). The site*sterilization interaction was significant for biomass of all species combined (Table 4.4), and all species alone except *Schizachyrium* (Table 4.5). Differences in biomass between sterilized and non-sterilized soils were less pronounced for Indiana compared to Crooked Creek and Spindletop soils (Fig. 4.9b). Within Indiana and Spindletop, the effect of sterilization on biomass was greatest in the forest stand soils. Seedlings from the fescue soils at Spindletop had significantly lower biomass in the non-sterilized soil compared to the sterilized soils, but biomass was not different between sterilized and non-sterilized for fescue soils from Indiana (Fig 4.9b).

The multiple significant interaction terms of site, stand, species, and sterilization on the biomass dataset make interpretation complex. Therefore, I decided to run Proc GLM on biomass for each species alone by site to further evaluate the main effects of stand, sterilization, and the interaction of these two main effects. Using this approach, for *Chamaecrista,* biomass was significantly affected by sterilization only at all sites, in all cases being significantly greater for non-sterilized compared to sterilized soils (Fig. 4.5a-c). The stand*sterilization and site*sterilization interactions detected in the full statistical model for this species (Table 4.5) are apparent in the by site data as differences in

biomass across stand types varies by sterilization treatment in site-specific ways (Fig. 4.5a-c).

Stand, sterilization, and the interaction were significant for *Echinacea* biomass at IN and CC, but at ST the interaction was not significant (Fig. 4.6a-c). In the non-sterilized soils, E+ had significantly higher biomass than forest soil from CC (Fig. 4.6b), but for ST soils, biomass from E+ soil was significantly lower than the forest soil (Fig. 4.6c). Patterns in biomass were similar at IN and ST, with the sterilized forest soils having higher biomass than either of the sterilized fescues (Fig. 4.6a,c), but this was not the case at CC (Fig. 4.6b). There the E- sterilized soils had higher biomass than either of the other stands. Differences in biomass between sterilized and non-sterilized were less obvious for Crooked Creek soils (Fig. 4.6b) compared to the other two sites (Fig. 4.6a,c). Biomass of this species in non-sterilized soil was generally low, and varied less between stand types and across sites than in the sterilized soil (Fig. 4.7a-c).

Panicum biomass from Indiana soils was only significantly affected by sterilization (Fig. 4.7a), while stand and the interaction with sterilization was significant at the other two sites (Fig. 4.7b,c). *Panicum* had the greatest response in biomass to sterilization of all the species, growing nearly 3.5x larger in sterilized soil (Fig. 4.9a). This increase in biomass following sterilization appeared to be greater for Spindletop (Fig. 4.7c) compared to IN (Fig. 4.7a) or CC (Fig. 4.7b). Of the sterilized soils, those from Spindletop exhibited the only significant differences between stand, with forest>E->E+ (Fig. 4.7c). In non-sterilized soils from both CC and ST, the forested soils supported significantly higher biomass than the fescue soils (Fig. 4.7b,c).

Within each site, *Schizachyrium* biomass was affected only by sterilization (Fig. 4.8a-c) (1.8x greater for sterilized than non-sterilized soil). Like *Echinacea*, differences in biomass of *Schizachyrium* seedlings between sterilized and non-sterilized soils appeared to be less dramatic in CC soils (Fig. 4.8b) compared to soils from the other two sites (Fig. 4.8a,c).

Mycorrhizal Colonization

Plant species significantly (Table 4.4) affected root mycorrhizal colonization (MC), with *Panicum* having the highest (38.02±1.24% of roots colonized when averaged across site, stand, and sterilization treatments), *Schizachyrium* the lowest (11.72±0.88%), and *Echinacea* intermediate (33.92±1.59%) (all were significantly different from each other). Site had a significant effect on MC for all species combined (Table 4.4), and for each species individually (Table 4.6). For all species combined and averaged across sterilization treatment, seedlings grown in Crooked Creek soil had the highest MC, Spindletop soil had the lowest MC, and Indiana soil was intermediate (all were significantly different) (Fig. 4.4b), although this effect was most pronounced in the fescue soils. Stand had a significant effect on MC for all species individually (Table 4.6) and combined (Table 4.4). In all cases, seedlings from the forest stand soils had significantly lower MC than either of the fescue soils, and MC from E+ and E- soils never significantly differed (Fig 4.4b). When analyzed by site for each of the species, MC very rarely differed between E+ and E- stand soils, and when it did (Fig. 4.6d, Fig. 4.8e) biomass did not differ for the same soils (Fig. 4.6a, Fig. 4.8b). Sterilization significantly reduced MC for all species individually (Table 4.6) and combined (Table 4.4). Sterilization reduced MC to the lowest levels in *Schizachyrium* (Fig. 4.8d-f) at only 1% of roots being colonized, compared to 8% root colonization for *Echinacea* (Fig. 4.6d-f) and 24% for *Panicum* (Fig. 4.7d-f) grown in sterilized soils.

As with biomass, because species and its interactions with site, stand, and sterilization significantly affected MC in a number of cases, I ran Proc GLM for MC of each species separately by site for the effects of stand, sterilization, and the interaction. For *Echinacea* both stand and sterilization and their interaction had significant effects within all sites (Fig. 4.6d-f). For non-sterile soils, MC of seedlings from the forest stand soils was lower than the fescue soils at all sites. However, with sterilization, this stand trend disappeared. For sterile soils from CC and ST, there were no significant differences for MC between stand types (Fig. 4.6e,f) but for sterile IN soils, the E- stand

resulted in significantly higher MC than E+ or forest (Fig. 4.6d). *Panicum* MC was affected only by sterilization at Indiana and Spindletop, but in Crooked Creek soils, stand was significant as well (Fig. 4.7d-f). The reduction in MC for *Panicum* in sterilized soils was less for CC (Fig. 4.7e) than the other two sites for this species (Fig. 4.7d,f). MC of *Schizachyrium* in IN soils was affected by stand and sterilization but not their interaction (Fig. 4.8d), being significantly lower in forested compared to fescue stands regardless of sterilization treatment. For non-sterile soils from CC, MC of *Schizachyrium* was significantly greater for seedlings from E- compared to E+ or the forest stand (Fig. 4.8e), but sterilization removed this stand effect. For ST soils, there was no difference between E+ and E- (Fig. 4.8f) stands in either sterilization treatment.

Statistical tests of arbuscule and vesicle abundance in my data revealed the same general patterns as overall mycorrhizal colonization (data not shown). Vesicle abundance was significantly affected by stand (p=0.0109), with fewer observed in forested compared to fescue soils (E+ and E- were not different from one another). Arbuscule abundance was not affected by stand, and was overall much lower than vesicle abundance (64 total out of 29,400 views, compared to 804 vesicles).

Relationship Between Mycorrhizal Colonization & Biomass

A regression performed on data from all species (the three for which MC was measured), sites, stands, and sterilization treatments indicated that mycorrhizal colonization was significantly correlated with plant biomass (p<0.0001, R^2=0.03, F=33.68). However, this relationship, while significant, was quite weak (low R^2) and was opposite that expected- biomass was negatively correlated with MC. When linear regressions were done for biomass and MC of non-sterilized and sterilized soils separately, the two were not significantly correlated for non-sterilized soils (p=0.24, R^2=0.003, F=1.41), but were significant for sterilized soils (p<0.0001, R^2=0.16, F=91.84). When compared to the R^2 for the whole data set, the higher R^2 for the sterilized soils,

and the lower R^2 for the non-sterilized soils indicates the significant negative relationship of biomass and MC is being driven largely by the sterilized soils.

I performed linear regressions of biomass and MC for each species to see if the significant negative relationship seen for all species held true across species or was being driven by certain species. The relationship was significant for all species (p<0.0001) and remained negative in all cases, but differed in its strength (R^2) between species. For *Echinacea* the regression explained 22.7% of the variance in the data, for *Panicum* it explained 29.7% of the variance in the data, and for *Schizachyrium* less than 1% of the variance was explained. Thus when looked at by species, *Echinacea* and *Panicum* appear to be primarily responsible for the significant negative relationship of biomass and mycorrhizal colonization, more so than *Schizachyrium*. This is not surprising given that *Schizachyrium* exhibited significantly lower mycorrhizal colonization compared to the other two species, apparently not forming associations as readily as *Echinacea* and *Panicum*.

Discussion

Did Mycorrhizal Colonization Differ Between the Stand Types or Soil Fertility?

I hypothesized that mycorrhizal colonization would be lowest in forest stand soils because forest soils tend to have fungal communities dominated by ectomycorrhizae (as opposed to endomycorrhizae), and I was testing for colonization by endomycorrhizae (AMF). I also predicted that MC would be higher in E- compared to E+ soils because prior work has suggested *Neotyphodium* presence in tall fescue plants reduces mycorrhizal spores and abundance in associated soils. In my experiment, I did measure significantly lower MC in forest stand soils compared to fescue soils across all three sites. However, there was no consistent difference for MC in seedlings grown in E+ and E- soils, regardless of whether soil had been sterilized or not. In the only two situations where differences in MC between E+ and E- were present in my study (*Echinacea*, IN, sterile and *Schizachyrium*, CC, non-sterile), MC was higher for E-

compared to E+ soils, which does correspond to my initial hypothesis on this topic; however, these differences were rare (observed in only two out of nine possible cases), and when present did not correspond with higher biomass of seedlings grown in E- vs. E+ soil. Although soil replicates for the initial soil microbial characterization effort were low (I only had a single composite sample from each site*stand combination), I did not observe significant differences in 16:1ω5, a common AMF biomarker, across stand types. Replicate samples within each site*stand combination may have clarified whether the AMF lipid biomarker varied initially by stand, as was observed in the MC data at the end of the experiment (FO<E+/E-). The few instances where MC differed between E+ and E- stands were surprising, given that other studies (Chu-Chou et al. 1992; Mack & Rudgers 2008; Antunes et al. 2008; Rudgers & Orr 2009) have suggested an allelopathic effect of E+ tall fescue on AMF.

Chu-Chou et al. (1992) measured reduced AMF spore densities in field soil beneath E+ compared to E- tall fescue stands, and Mack and Rudgers (2008) found AMF colonization of E+ fescue plants was reduced compared to E- plants. Rudgers and Orr (2009) did not directly measure AMF abundance or colonization, but they saw depressed growth of some native tree seedlings under non-sterilized E+ soil compared to E-, which they attribute to alteration of soil microbial communities, and likely AMF. Antunes et al. (2008) observed a reduction in arbuscules and an increase in vesicles for plants watered through E+ litter compared to E-, but the total percentage colonization was not that different and was not statistically analyzed. I did not observe a significant difference in arbuscules or vesicles between E+ and E- soils in my study. It may be that E+ fescue suppresses certain AMF species not present or overly abundant in my study. My results suggest that within the context of grassland restoration and for the plant species I studied, endophyte presence within the tall fescue community did not have consistent, strong negative impacts on the degree of mycorrhizal associations formed.

Soil fertility, and in particular P, varied between stands and sites. Given the importance of P in AMF abundance and colonization, I had hypothesized that MC

would be highest in the lowest P soils, and lowest in the highest P soils. This prediction was verified: Crooked Creek had the lowest P and highest MC of the sites, and Spindletop had the highest P and lowest MC. P was significantly greater in forest stand soils compared to fescue soils, and MC was significantly lower in the forest compared to fescue soils.

Increased P following steam sterilization has been measured in other studies (Seeling & Jungk 1996; Schmidt et al. 1997), as has an increase in plant biomass in response to this pulse of available P and/or N (Jakobsen & Anderson 1982; Meredith & Anderson 1992), and both of these effects were observed in my study. The effect of sterilization on MC (a decrease) was less for CC than the other two sites, which may reflect the low P of this site. Although sterilization increased P in the soil of this site, P concentrations were still much lower in these CC soils compared to the other two sites. Therefore, the seedlings in CC sterilized soils may have been forming associations more readily than at the other two sites in a continued effort to acquire adequate P. On the other hand, site and stand differences in MC cannot be entirely linked to P availability, as AMF abundance may have been lower in the forest stand soils to begin with (although not detected by lipid biomarker in our limited PLFA dataset). Furthermore, the effect of sterilization on P levels was equal across all soils, but differences in MC between sterilized (higher P) and non-sterilized (lower P) soils varied between stands within sites and by species, indicating factors other than P availability influenced MC.

Were Microbial Symbionts Beneficial to Seedlings as Reflected by Biomass?

I hypothesized that microbial symbionts would be beneficial to seedlings, resulting in higher biomass in non-sterilized soils compared to sterilized, as prior research has shown all species (or genera) used in this experiment form and seem to benefit from AMF associations (*Echinacea:* Wilson & Harnett 1998; Lata et al. 2003; Araim et al. 2009*, Panicum*: Brejda et al. 1998; Johnson 1998; Ghimire et al. 2009, *Schizachyrium*: Burrows & Pfleger 2002), and association with AMF is considered

important for growth of many plants in grassland ecosystems (Anderson et al. 1984; Miller 1987; Wilson & Harnett 1998). This response might also be reflected by a positive correlation of biomass and MC across the species. These hypotheses were not supported by my study- the only species in which biomass was higher in non-sterilized soils was for the N-fixer *Chamaecrista.* For this species, the benefit of symbiosis with soil microbes, and in particular N-fixing rhizobia (evident by nodules on harvested roots, more flowers, and darker green color compared to plants grown in sterilized soils), apparently outweighed any benefit in growth associated with the increased pulse of P resulting from sterilization. In non-sterile soil, *Chamaecrista* had lower biomass in FO compared to fescue soils, where presumably rhizobia are less abundant (Nüsslein & Tiedje 1999). The other three plant species had lower biomass in non-sterilized compared to sterilized soils. This unexpected response may indicate that these species were apparently not benefiting from microbial symbionts in non-sterilized conditions, and/or possibly experiencing a negative effect from other soil microorganisms (pests and/or pathogens) present in the non-sterilized soils, and/or experienced stimulated plant biomass due to the increased P resulting from soil sterilization (having nothing to do with soil microbes). Whatever the reason(s), the increase in biomass for these species in sterilized soils resulted in a significant negative correlation for biomass and MC for all species combined.

A negative effect of soil microorganisms on growth could include a parasitic effect of AMF on the host plant. This has been clearly demonstrated for *Schizachyrium* (Meredith & Anderson 1992; Hartnett & Wilson 1999), but for the other species included in this study (or plants from the same families), AMF have mostly been found to be beneficial to growth. Other microorganisms (e.g. nematodes, viruses, etc.) may have played a role in depressing plant growth in the non-sterilized soils, which could explain why some species experienced greater growth release following sterilization. Of the three species, *Panicum* experienced the greatest increase in growth in sterilized compared to non-sterilized soils, and the increase appeared to be greatest in the

forested stand soils, despite plants from those soils having lower MC compared to the fescue stands. This result does not support a parasitic effect of AMF alone, which would result in the largest biomass increase for the plants that had the highest MC (in the fescue soils). Limits to growth by microorganisms present in the non-sterilized soils could easily be expected to vary between different site and stand soils, and to affect plant species differently. This possible explanation for the sterilized vs. non-sterilized soil effect on biomass may better explain the observed variability than increasing P availability alone. Given multiple possibilities explaining the sterilization effect on biomass, I am unable to conclude whether a negative impact of soil microorganisms or lower P are responsible for the lower biomass in non-sterilized soils, but it seems likely that both may have played a role.

Additional evidence for a lack of AMF symbiosis benefit to plant biomass comes from the linear regressions run for non-sterilized soils only: biomass and MC were not significantly correlated. When analyzed for each of the three species alone, the relationship remained significant and negative in all cases. In this study, association with AMF did not appear important for plant growth in any of the three species examined for MC, at least not within the first two months of growth. It could be that I harvested plants during an initial "transient depression" (Koide 1985), when the effects of AMF were largely parasitic, but had I given the seedlings a longer period of time to grow, the associations would have become beneficial. Other greenhouse studies have used a longer growth period (ie. ~3 mos. Meredith & Anderson 1992; Brejda et al. 1993; Brejda et al. 1998; Lata et al. 2003; Vatovec et al. 2005; Araim et al. 2009, 4 mos. Wilson & Harnett 1998; Klironomos 2003, 5 mos. Pringle & Bever 2008; Wilson et al. 2001), but two months has been a long enough period to result in enhanced biomass attributed to MC in at least some cases (Koide et al. 2000; Ghimire et al. 2009). Previous research indicating differences in growth resulting from AMF associations within the time period of this study and with the same plant species point to something other than harvest during a period when AMF were parasitic on these species as driving the

observed response. Given that abundance of and/or association with AMF varies depending on abiotic conditions such as soil nutrients (Anderson et al. 1984; Anderson et al. 1994; Eom et al. 1999; Gai et al. 2009; Hoeksema et al. 2010; Johnson et al. 2010) and soil moisture (Anderson et al. 1984; Jones & Smith 2004), it may be that the growing conditions in this study were such that AMF associations were not necessary or favorable to growth. It is possible that even though associations formed, they did not enhance host plants because beneficial exchange of nutrients and water between AMF and the host plant (Harley & Smith 1983) may have been at low rates due to favorable abiotic conditions (soil fertility and/or moisture). Hetrick et al. (1990) manipulated soil P levels and examined MC of grasses, and saw low levels of MC persisting even when association did not have an effect on growth (when P levels were high). Despite the wide range of P levels we measured between different stands and sites, we never observed a positive effect of MC on biomass, even in the lowest P soils.

How did Mycorrhizal Colonization Differ Between the Species?

I had predicted that there would be differences in the level of MC between the species, reflective of their reliance on AMF. Of the three species, previous studies have found *Schizachyrium* associations may result in no response (Landis et al. 2005), positive growth (Burrows & Pfleger 2002), or negative growth (Meredith & Anderson 1992; Hartnett & Wilson 1999). Anderson (2008) found no differences in MC of this species between grasslands restored two and twelve years prior to the study, and no correlation for biomass and MC or link to P levels. Gustafson and Casper (2004) looked at *Schizachyrium*, *Andropogon gerardii*, and *Sorghastrum nutans* grown in different soils and with different nutrient levels, and found no difference in growth for *Schizachyrium*, as with the other two species. Bingham and Biondini (2009) on the other hand, observed positive correlation of hyphal length and plant biomass for this species, and they lump it in with *Panicum virgatum, Andropogon gerardii,* and *Sorghastrum nutans* as an obligatory mycorrhizal species. In my study, *Schizachyrium* formed the lowest levels of MC for the three species. In sterilized soils, MC for this species was nearly zero, but

plants still established well despite no association with mycorrhizae. This species has also exhibited the ability to effectively establish early in restorations compared to other native grasses (Washburn et al. 2000). Given that this species is the dominant grass in current day native grassland ecosystems within Kentucky and the region (Chester et al. 1997; Jones 2005) and that it does not seem to be dependent on AMF, which can be limited in abundance in disturbed soils (Li et al. 2007), *Schizachyrium* may be a good species to use during early restoration or renovation of native grassland in many different soil types.

Evidence of mycorrhizal dependence for *Panicum* is more consistent and well established than for *Schizachyrium*. Clear positive growth responses of *Panicum* following association with AMF have been documented (Brejda et al. 1998; Johnson 1998; Ghimire et al. 2009), with only one case of a neutral response (Hartnett et al. 1994) (and none negative). In my study, *Panicum* had significantly higher MC compared to the other species, and MC was high even in the sterilized soils. This seems to indicate a reliance of this species on AMF, and for reasons other than P, given that sterilized soils (and soils from ST) had higher P but associations were still formed. It is therefore surprising that this species, like the others in this study, exhibited a negative relationship between MC and plant biomass. Unlike *Schizachyrium*, *Panicum* is rarely observed in restorations in Kentucky without purposeful seeding, and even when planted, does not establish as well as *Schizachyrium* (Washburn et al. 2000), which may in part, be due to its reliance on AMF, if AMF abundance is low in the initial soils.

Echinacea spp. has been shown to respond positively in growth to AMF (Wilson & Hartnett 1998; Araim et al. 2009; Lata et al. 2003), with no cases of negative growth. In my study, *Echinacea* had MC that was intermediate of the two grass species. This was true in both sterilized and non-sterilized soils. Mycorrhizal colonization was fairly consistent across sites, despite large variability in soil fertility. MC in this species is apparently independent of soil P. Biomass for *Echinacea* seedlings was not positively

correlated with MC, so as with both of the other species, although associations formed in these seedlings they did not enhance growth.

Mycorrhizal fungi are clearly an important and complex component of the soil microbial community (Lekberg & Koide 2005; Johnson et al. 2010; Klironomos et al. 2011) that can have strong effects on the plant community (van der Heijden et al. 1998; Brundrett 2002). As with other microbial symbionts (e.g. fungal endophytes), the influence that they have on host plants may depend not only on fungal species (Bennett & Bever 2007; Maherali & Klironomos 2007), but also fungal strain (Clark et al. 1999; Clark 2002) and host plant cultivar/genotype (Koslowsky & Boerner 1989; Brejda et al. 1998; Cavender & Knee 2006). My study did not explore any of these possible effects. Many studies use fungal inoculants of a single or a handful of AMF species (e.g. Anderson et al. 1994; Lata et al. 2003; Araim et al. 2009; Liu et al. 2011) which may represent a fungus/fungi selected specifically for its/their likelihood to demonstrate a positive growth response in the host plants chosen. The use of sterile growth medium supplied with specific inoculants removes the influence of other microorganisms (including those that may act as pests or pathogens) that may be important in soils. My approach in this study treated the soil microbial community as a "black box," using soil that had either all (non-sterilized) or none (sterilized) of the soil microbial communities present. This approach seems most useful in the context of restoration, as the purposeful sterilization and subsequent addition of a specific inoculum across large areas is impractical, but it does not allow for detection of effects from specific microorganisms, interactions between microorganisms, or plant-microorganism interactions. Thus, I am unable to say exactly why I did not observe a positive biomass response to AMF in this study, but in addition to the abiotic factors discussed earlier, these biotic factors could explain these differences in my results compared to others.

Conclusions

There were no consistent, significant differences in mycorrhizal colonization for the three species examined when grown from soil beneath E+ and E- tall fescue, nor was biomass depressed in these plant species between these two stand types. These results indicate that the presence of an aboveground fungal endophyte within the tall fescue community does not suppress AMF or appear to produce other allelopathic effects on the seedlings of the native grassland species utilized in this study. My results indicate that fungal endophyte presence/abundance in tall fescue pastures slated for restoration/renovation is unlikely to be a major factor in dictating the success of these efforts. There was evidence for the influence of site and stand type trends in soil P on mycorrhizal colonization and growth in this study; however there was also evidence that other microorganisms (pests/pathogens) played a role in determining seedling biomass, given the dramatic but variable increase in growth for three of the four species in sterilized soils across sites and stand types. Finally, differences in mycorrhizal colonization varied among species, but none exhibited the predicted positive relationship with biomass. Instead the opposite was observed. *Schizachyrium* grew relatively well in this study, despite extremely low mycorrhizal colonization in sterilized soils, and it also grew well in non-sterilized soils, indicating this species does not rely on AMF and/or may tolerate microbial pests/pathogens that negatively impacted the growth of the other species. These results suggest this species may be a good choice for grassland restoration, when the abundance of AMF and other soil microorganisms is unknown.

Tables

Table 4.1. Tall fescue percent cover and endophyte infection frequencies (given in parentheses) where replicate soil samples (A-D) were collected from each site. Percent cover was averaged for the two 0.25 x 0.25m quadrats per replicate and endophyte infection frequencies were from the composited fescue tillers sampled per replicate (6 from each quadrat).

	Indiana		**Crooked Creek**		**Spindletop**	
Sample	**E+**	**E-**	**E+**	**E-**	**E+**	**E-**
A	91.5(100)	65(27)	75(100)	75(0)	96.5(100)	80(0)
B	91(100)	80(0)	74(75)	40(0)	99(100)	93.5(0)
C	95(91.7)	77.5(0)	86(91.7)	64(0)	97.5(91.7)	99(0)
D	98(91.7)	94(0)	97.5(100)	59(0)	98(69.2)	97.5(0)

Table 4.2. P-values for the main effects of site, stand, and their interaction on soil parameters for non-sterilized soils used in greenhouse experiment. NS indicates p-values >0.05.

	Total N (%)	**Total C (%)**	**P (mg/kg)**	**K (mg/kg)**	**Ca (mg/kg)**
Site	0.0128	0.0002	<.0001	<.0001	0.0029
Stand	<.0001	<.0001	0.0172	NS	0.0023
Site*Stand	0.0327	NS	0.0195	NS	0.0006

Table 4.3. Means (±1 SE) for soil parameters between stands within sites, across all sites, and across all stands (non-sterilized only). Lowercase letters represent LS Means differences for the main site effect in "Site Average" panel, the main stand effect in the "Stand Average" panel, and the Site*Stand effect when significant in panels "IN", "CC", and "ST". Uppercase letters represent LS Means differences between stands within a site when the Site*Stand effect was not significant.

	IN			CC			ST			Site Average			Stand Average		
	E+	E-	FO	E+	E-	FO	E+	E-	FO	IN	CC	ST	E+	E-	FO
Total N (%)	0.16d (±0.02)	0.19cd (±0.03)	0.36ab (±0.03)	0.19cd (±0.03)	0.30b (±0.03)	0.36ab (±0.04)	0.19cd (±0.00)	0.21c (±0.01)	0.44a (±0.01)	0.24b (±0.03)	0.29a (±0.03)	0.28a (±0.04)	0.18c (±0.01)	0.23b (±0.02)	0.39a (±0.02)
Total C (%)	1.41B (±0.16)	1.72B (±0.28)	4.18A (±0.34)	1.95C (±0.24)	3.17B (±0.26)	5.14A (±0.58)	1.76B (±0.03)	1.9B (±0.10)	4.97A (±0.17)	2.50c (±0.43)	3.55a (±0.46)	2.87b (±0.45)	1.68c (±0.10)	2.31b (±0.23)	4.76a (±0.24)
P (mg/kg)	21.25de (±6.44)	28.18cd (±10.37)	55.38c (±15.68)	19.0de (±4.51)	8.63e (±1.42)	7.88e (±0.85)	125.00b (±11.55)	144.00b (±13.98)	208.25a (±8.16)	35.55b (±7.78)	11.18c (±1.93)	159.08a (±12.30)	58.36b (±16.55)	63.18b (±20.16)	90.50a (±26.32)
K (mg/kg)	172.13A (±26.09)	164.00A (±42.65)	166.38A (±23.08)	246.82A (±50.76)	250.13A (±6.96)	214.88A (±11.20)	101.00A (±11.23)	116.13A (±27.97)	158.25A (±17.35)	167.82b (±15.26)	236.41a (±13.65)	125.13c (±12.78)	166.64a (±23.7)	177.91a (±22.88)	179.83a (±12.0)
Ca (mg/kg)	1629.38e (±254.8)	1905.83cde (±323.55)	3824.38a (±328.42)	1903.50cde (±519.2)	2646.25cd (±359.6)	1608.38e (±355.5)	2607.00c (±151.3)	2781.75bc (±203.3)	3626.38ab (±230.0)	2503.0b (±354.3)	2066.3b (±251.3)	3005.0a (±169.3)	2059.b (±206.3)	2493.6b (±193.9)	3019.7a (±342.4)

Table 4.4. P-values for effects of site, species, stand, sterilization, and all interactions on biomass and mycorrhizal colonization (MC). NS indicates p-values >0.05.

	Biomass	**MC**
Site	<0.0001	<0.0001
Species	<0.0001	<0.0001
Site*Species	<0.0001	<0.0001
Stand	NS	<0.0001
Site*Stand	0.0009	NS
Stand*Species	<0.0001	NS
Site*Stand*Species	NS	NS
Sterilization	<0.0001	<0.0001
Site*Sterilization	<0.0001	NS
Species*Sterilization	<0.0001	<0.0001
Site*Species*Sterilization	0.0009	<0.0001
Stand*Sterilization	0.0001	<0.0001
Site*Stand*Sterilization	<0.0001	NS
Stand*Species*Sterilization	<0.0001	0.0127
Site*Stand*Species*Sterilization	NS	NS

Table 4.5. P-values for effects of site, stand, and sterilization treatments and their interactions on total biomass of seedlings by species. NS indicates p-values >0.05.

	Chamaecrista	***Echinacea***	***Panicum***	***Schizachyrium***
Site	0.0263	0.0121	0.0005	0.0024
Stand	0.0147	NS	<0.0001	NS
Sterilization	<0.0001	<0.0001	<0.0001	<0.0001
Site*Stand	0.0036	0.0003	0.0006	NS
Stand*Sterilization	<0.0001	0.0377	NS	NS
Site*Sterilization	0.0147	<0.0001	<0.0001	NS
Site*Stand*Sterilization	NS	0.0013	0.0115	NS

Table 4.6. P-values for effects of site, stand, and sterilization treatments and their interactions on mycorrhizal colonization of seedlings by species. NS indicates p-values >0.05.

	Echinacea	*Panicum*	*Schizachyrium*
Site	0.0440	0.0003	0.0278
Stand	<0.0001	<0.0001	0.0166
Sterilization	<0.0001	<0.0001	<0.0001
Site*Stand	NS	<0.0001	NS
Stand*Sterilization	<0.0001	NS	<0.0001
Site*Sterilization	NS	0.0006	0.0294
Site*Stand*Sterilization	NS	NS	NS

Figures

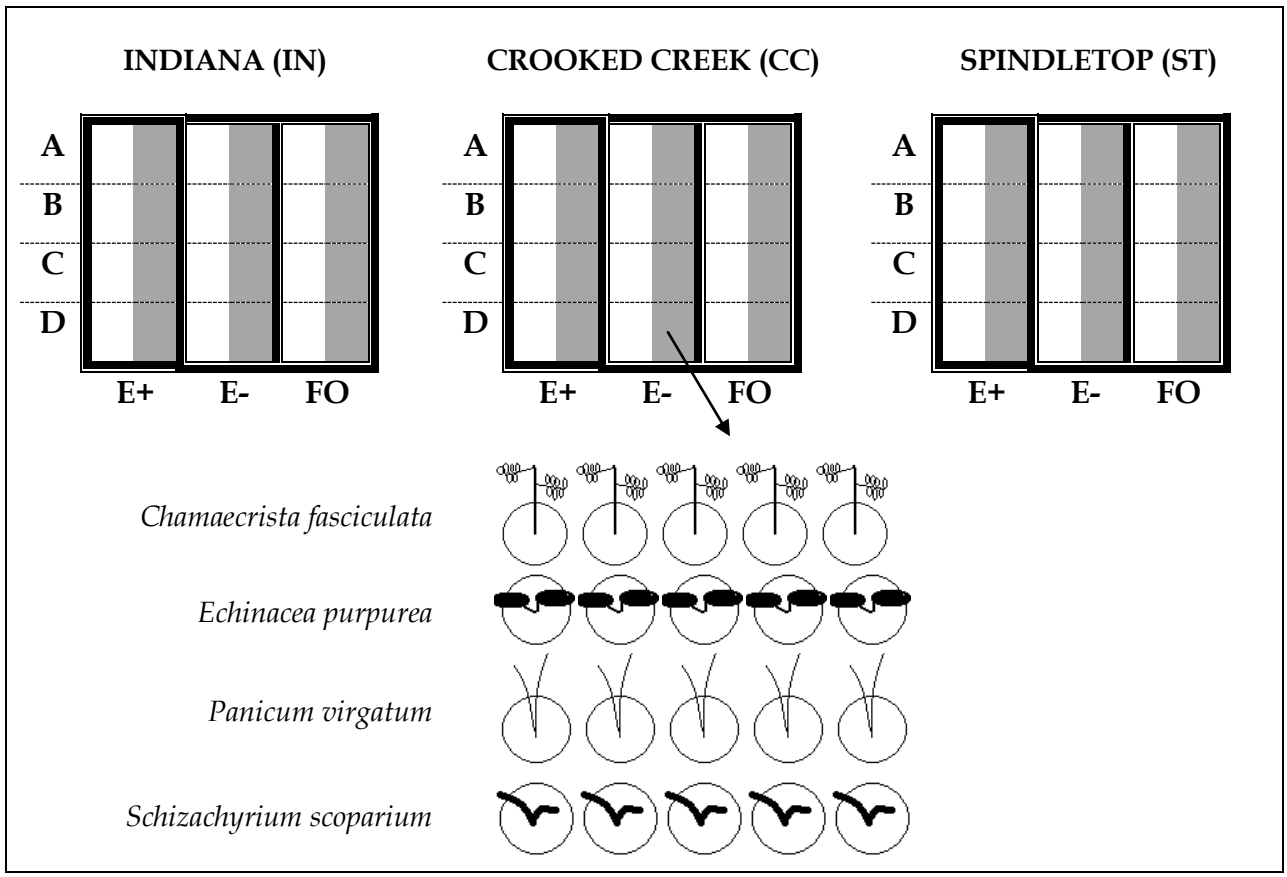


Figure 4.1. Schematic of experimental design. Thick lines separate each whole plot treatment or site*stand combination. Dotted lines separate the four replicate samples taken within each site*stand treatment. The shaded half of each of these represents the steam sterilization treatment. The bottom half of the figure depicts seeds planted into each site*stand*sample*sterilization soil (five replicates of each of four species).

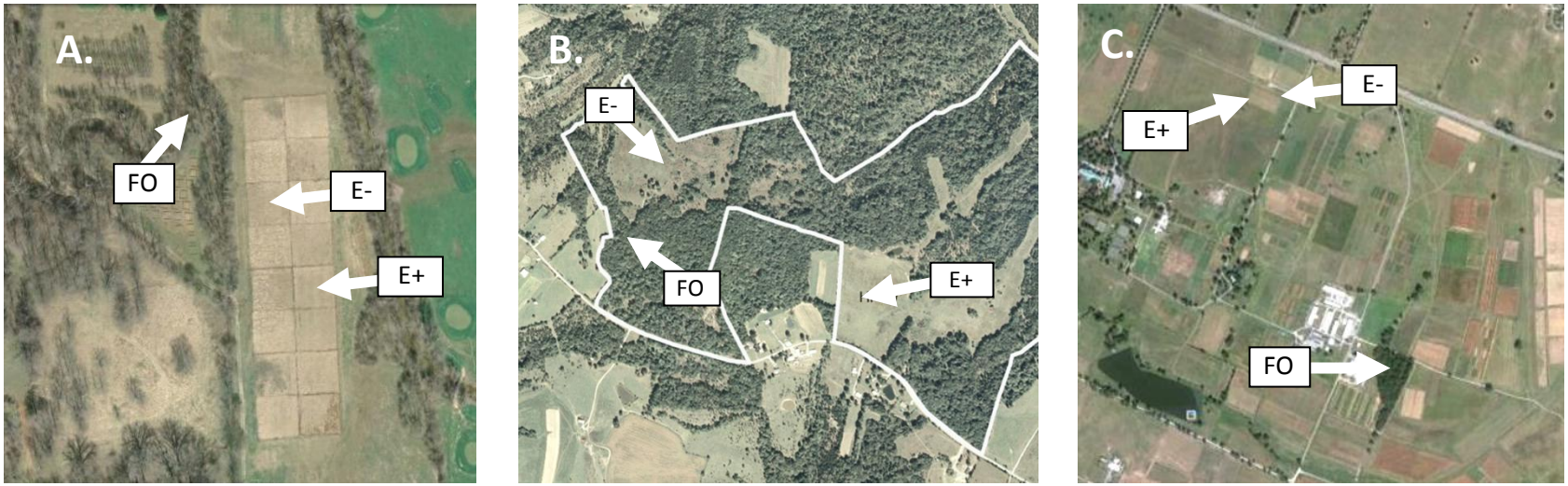


Figure 4.2. Aerial view of the three sites where soil was collected for this study: Indiana (a), Crooked Creek State Nature Preserve (b), Spindletop Research Farm (c). Sampling locations for soil from beneath endophyte-free (E-), endophyte-infected (E+) and forested (FO) vegetation are indicated.

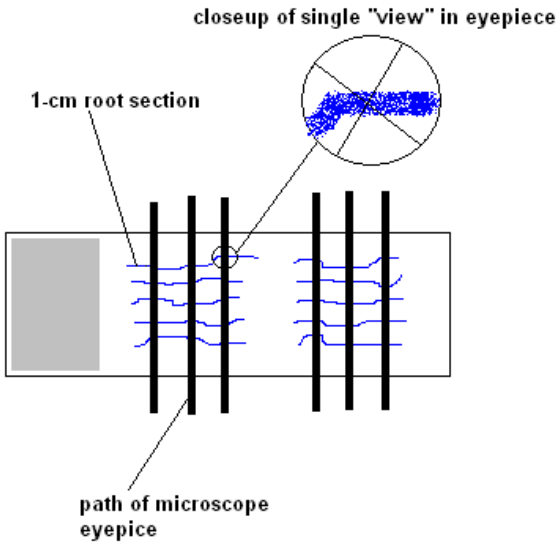


Figure 4.3. Schematic of microscopal slide scoring for mycorrhizal colonization.

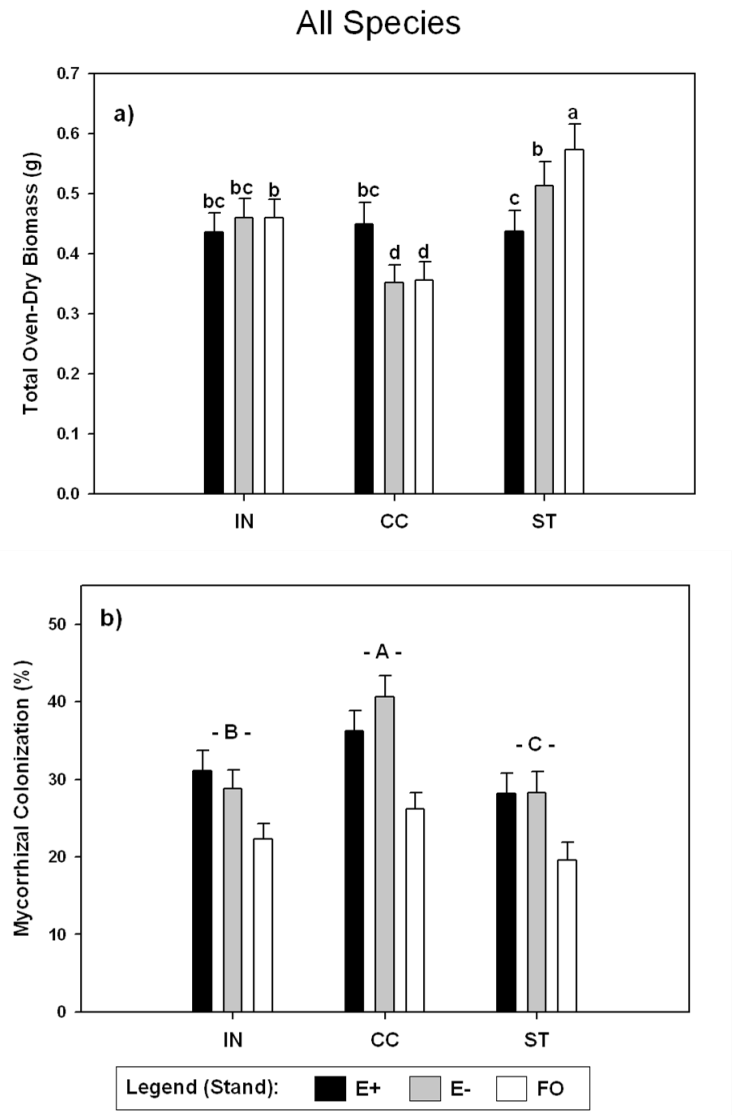


Figure 4.4. Mean biomass (a) and mycorrhizal colonization (b) of native grassland seedlings (four species combined) grown in soil from three different stand types (endophyte-infected (E+) and endophyte-free (E-) tall fescue and forest (FO)) within each of three sites (Indiana (IN), Crooked Creek (CC), and Spindletop (ST)), and averaged across sterilization treatment. Error bars indicate one standard error of the mean, and lowercase letters above error bars indicate significant differences as determined by LSMeans for the Site*Stand interaction when significant. Uppercase letters with dashes indicate significant differences as determined by LSMeans for the main Site effect when Site*Stand interaction was not significant (Table 4.4). For mycorrhizal colonization (b), stand had a significant effect (Table 4.4) such that E+/E->FO.

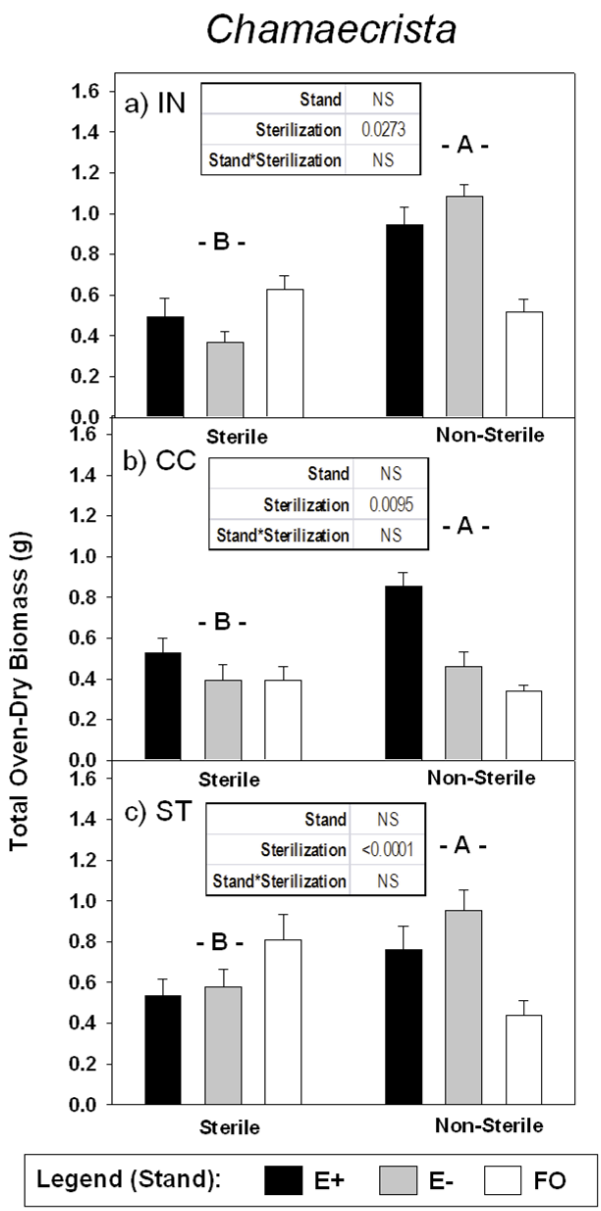


Figure 4.5. Mean biomass for *Chamaecrista* by sterilization treatment and stand within each site. Error bars indicate one standard error of the mean. P-values for the effects of stand, sterilization, and the interaction for each panel is presented. Uppercase letters indicate LS Means differences for the main sterilization effect.

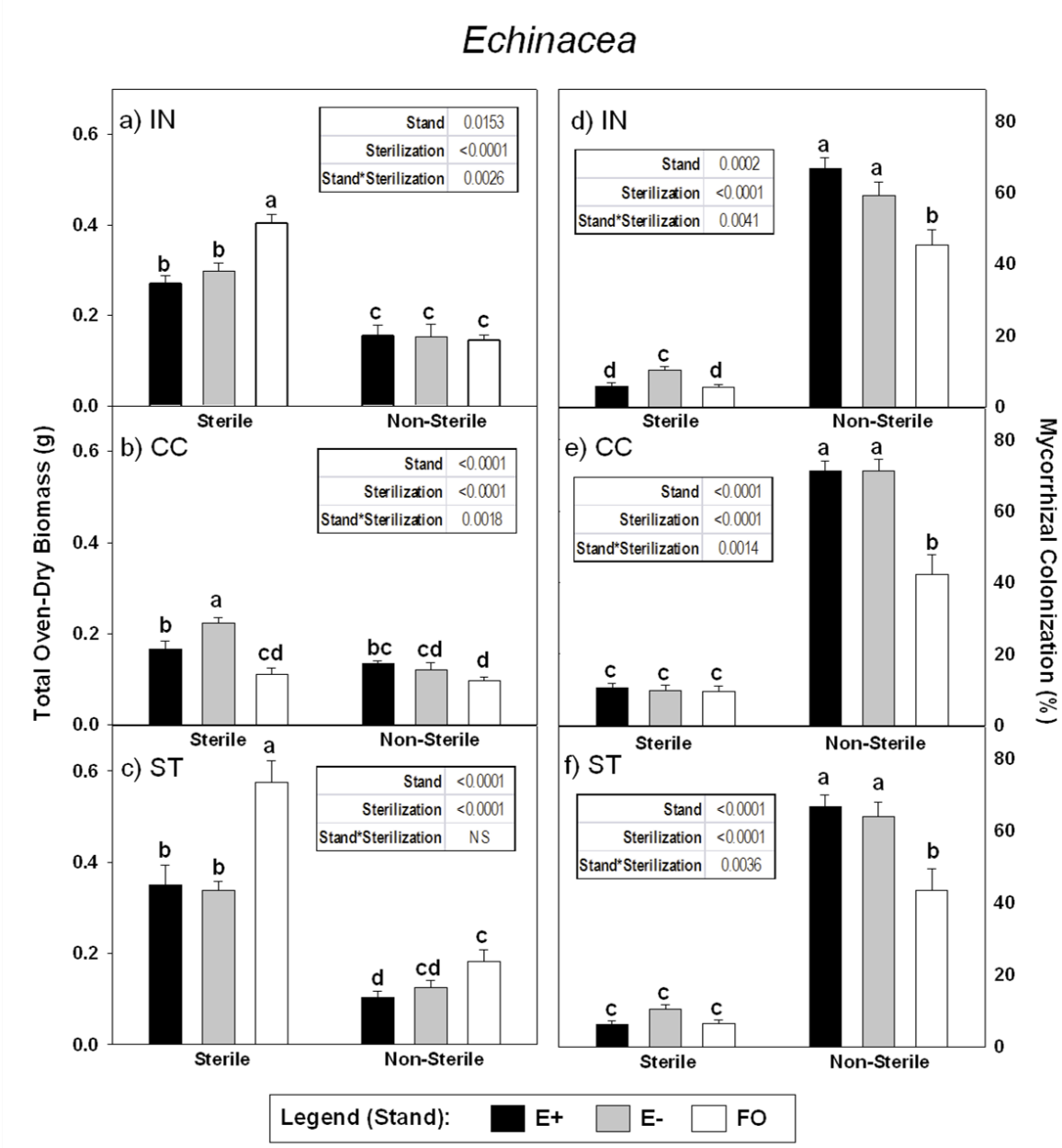


Figure 4.6. Mean biomass (a-c) and mycorrhizal colonization (d-f) for *Echinacea* by sterilization treatment and stand within each site. Error bars indicate one standard error of the mean. P-values for the effects of stand, sterilization, and the interaction for each panel is presented. Lowercase letters indicate LS Means differences for stand*sterilization when significant, uppercase letters indicate LS Means differences for the main sterilization effect when the interaction was not significant.

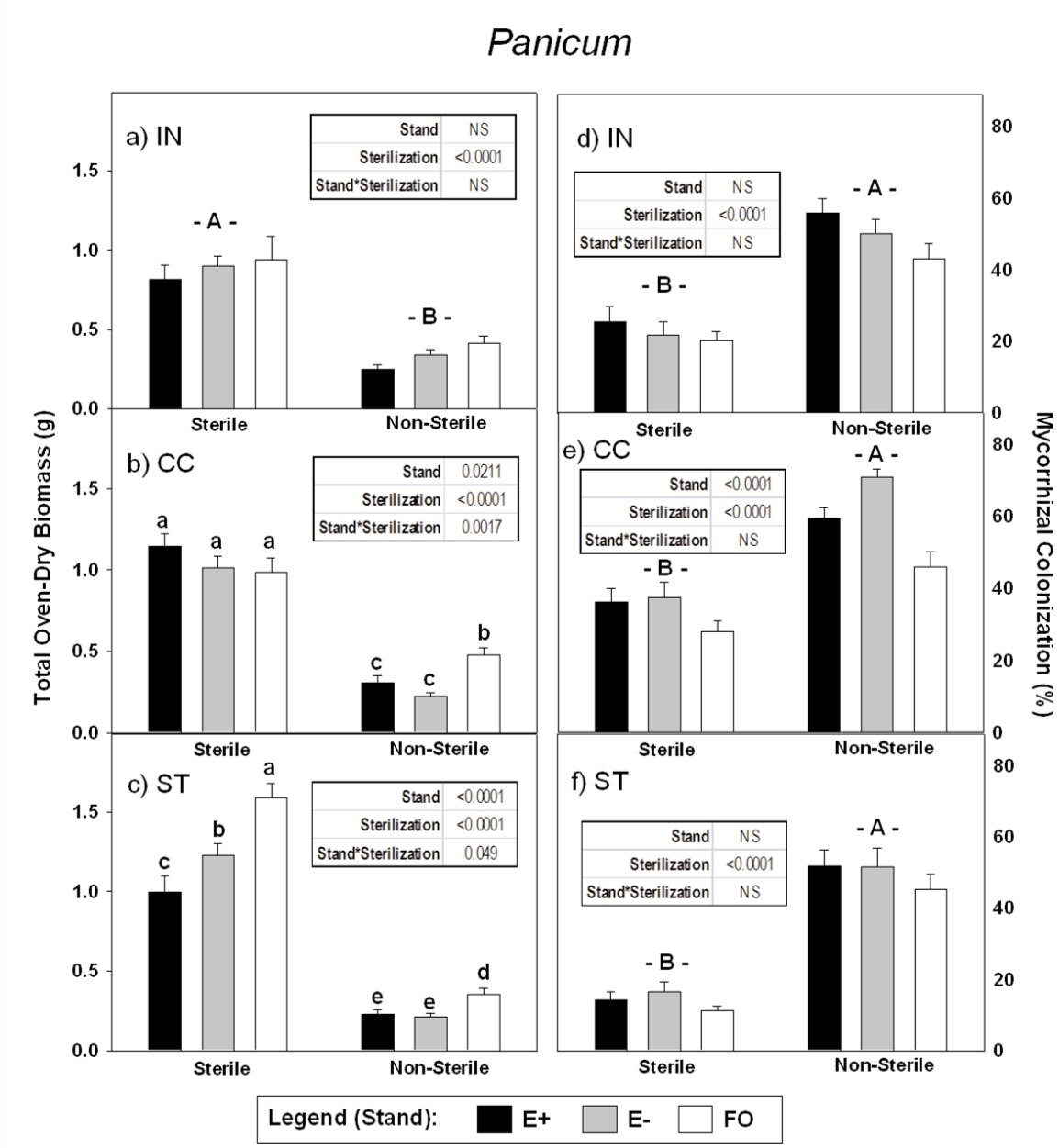


Figure 4.7. Mean biomass (a-c) and mycorrhizal colonization (d-f) for *Panicum* by sterilization treatment and stand within each site. Error bars indicate one standard error of the mean. P-values for the effects of stand, sterilization, and the interaction for each panel is presented. Lowercase letters indicate LS Means differences for stand*sterilization when significant, uppercase letters indicate LS Means differences for the main sterilization effect when the interaction was not significant.

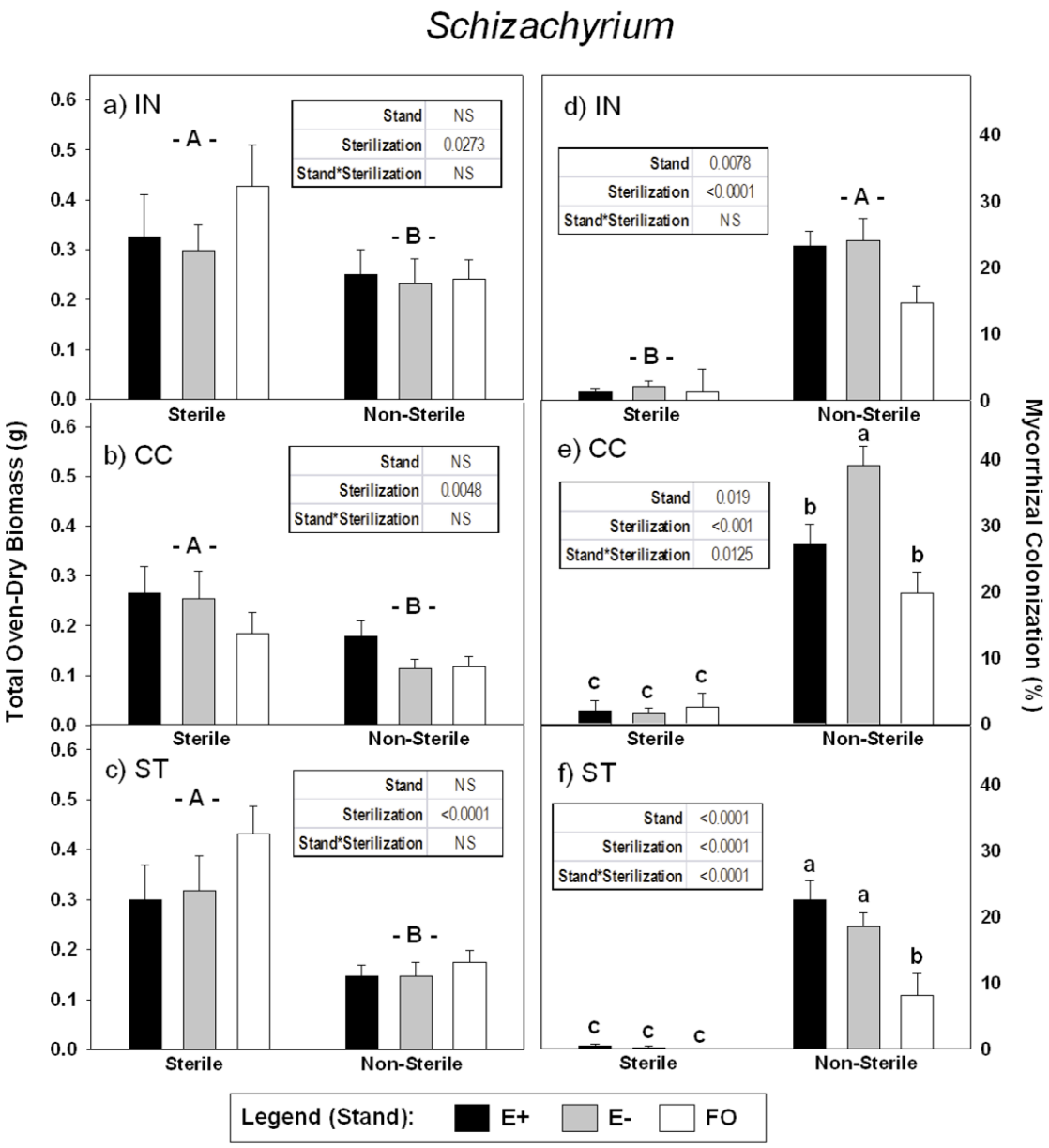


Figure 4.8. Mean biomass (a-c) and mycorrhizal colonization (d-f) for *Schizachyrium* by sterilization treatment and stand within each site. Error bars indicate one standard error of the mean. P-values for the effects of stand, sterilization, and the interaction for each panel is presented. Lowercase letters indicate LS Means differences for stand*sterilization when significant, uppercase letters indicate LS Means differences for the main sterilization effect when the interaction was not significant.

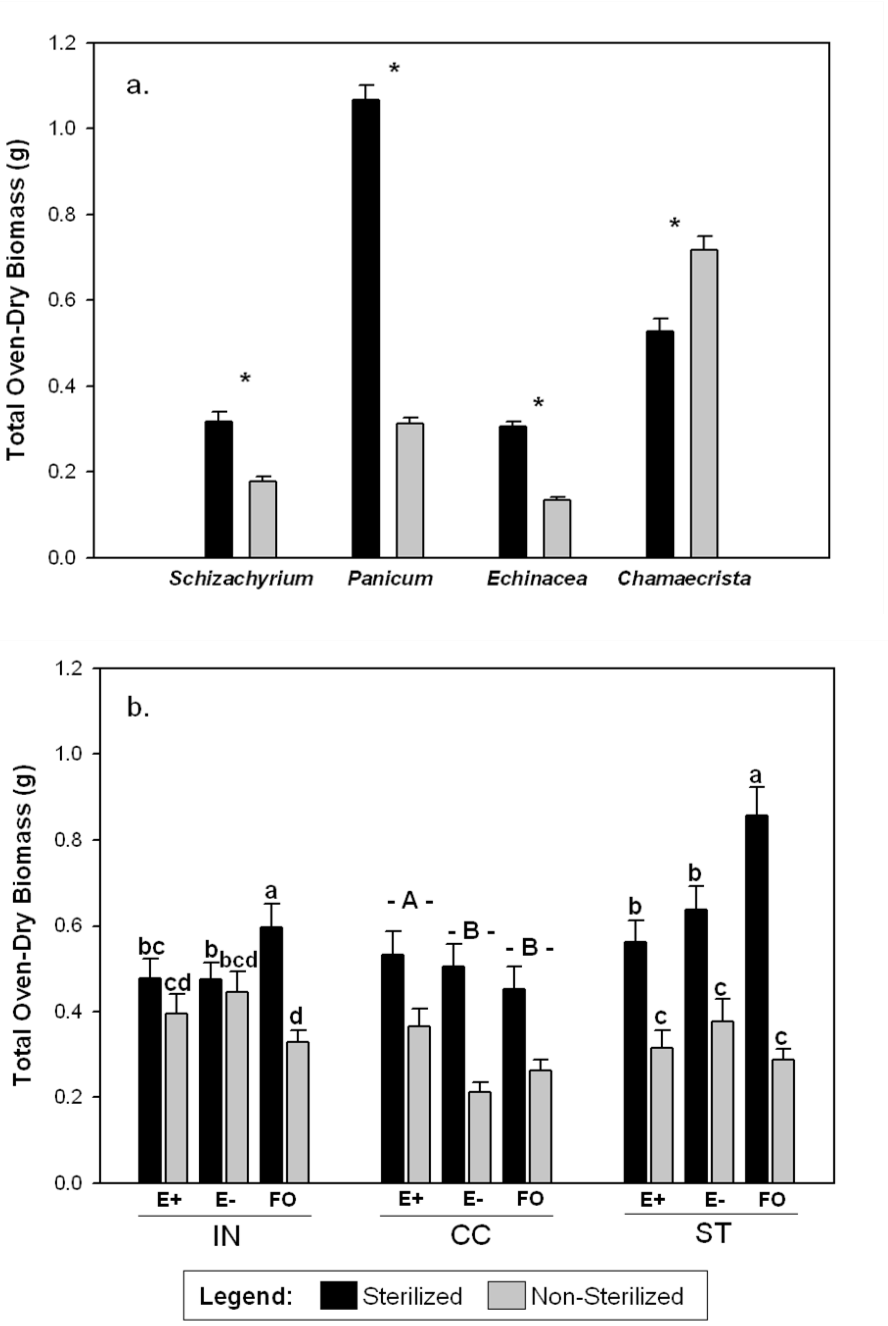


Figure 4.9. Mean biomass by sterilization treatment for each of the four plant species (a) and for the four species combined by site and stand (b). Error bars indicate one standard error of the mean. Asterisks above bars in indicate significant differences ($p<0.0001$) between means for sterilized and non-sterilized soils within a species (a). Lowercase letters indicate LS Means comparisons for the site*sterilization effect within a site when significant, and uppercase letters indicate the main site effect when site*sterilization was not significant (b).

Chapter Five

Conclusions

Project Summary

This research found little evidence to support the idea that the tall-fescue endophyte symbiosis will impact native, warm season grassland restoration success. I found no evidence for E+ plants preferentially surviving restoration management in the field (following selective herbicide application and/or prescribed burns) or greenhouse (prescribed burns), and little evidence of suppression of mycorrhizal fungi in the soil that form symbioses with planted native species. My results were surprising, given the widely accepted view that *N. coenophialum* provides fitness benefits in times of stress to those fescue plants that have it (Siegel & Bush 1994; Clay & Schardl 2002; Rodriguez et al. 2009). However, as scientists continue to conduct research on this topic, it is becoming clear that there is considerable complexity in the fungal endophyte-grass symbioses, and the abiotic and biotic factors that influence the effects of this symbiosis within the host plant (Saikkonen et al. 1998; Faeth & Fagan 2002; Faeth & Sullivan 2003).

Even when the full body of literature on the *N. coenophialum*-tall fescue interaction is examined, it is clear that the results are quite mixed, and when significant differences between E+ and E- plants exist, they are often relatively subtle or stand in complete contrast to the findings of similar studies. One example is the relationship of E+ tall fescue and plant diversity. Keith Clay and colleagues (Clay & Holah 1999; Rudgers & Clay 2007; Rudgers et al. 2007; Rudgers et al. 2010) have conducted a handful of experiments showing a negative relationship of endophyte infection rates of tall fescue on plant diversity, but Spyreas et al. (2001) observed a positive relationship of plant

diversity and endophyte infection frequency. Anti-herbivory properties conferred by *N. coenophialum* are another area of research in which the results are more mixed than often conveyed. Clear negative responses in insect bioassays have been demonstrated (Clay et al. 1985; Siegel et al. 1990; Bultman & Bell 2003; Salminen et al. 2005), but there is also evidence of either no response (Salminen et al. 2005) or positive response (Bultman & Bell 2003). Alkaloid concentration has been linked to soil fertility (Lyons et al. 1986; Arechavaleta et al. 1992; Saikkonen et al. 1998), drought stress (Arechavaleta et al. 1992), temperature/season (Belesky et al. 1988; Salminen et al. 2005), and plant genotype (Hill et al. 1991), all of which may play a role in determining whether anti-insect herbivore properties are present and their relative strength. Even when endophyte effects are present, the question for those interested in the symbiosis from a management standpoint would be whether or not there are implications in the field. Some have criticized endophyte studies for using insects for bioassays that wouldn't normally be problematic pests on tall fescue (Faeth & Bultman 2002), questioning the practical applications. The same questions could be asked in other cases where significant endophyte effects are seen. For example, Pecetti et al. (2008) saw 4.4% higher yield in E+ versus E- tall fescue, which was statistically different, but may have negligible implications in the field.

Impacts of the *Neotyphodium* endophyte on ecosystem processes also are unclear. Franzluebbers et al. (1999) first reported higher organic C and N beneath E+ tall fescue, but later (Franzluebbers & Stuedemann 2005) found higher C and N only for plots that received high levels of fertilization (no difference under low fertilization). In a 60-week mesocosm study, Franzluebbers (2006) found no differences in the soil C and N fraction due to endophyte presence. Siegrist (2008) observed significantly higher soil organic C and N for soils from E+ plots, although that difference did vary between the nine sites he looked at. In my

study (chapter four), there was a significant effect of stand type on %C and %N, but values were higher for E- than E+.

In my study, where differences were seen in E+ and E- tall fescue (growth parameters in chapter three), *N. coenophialum* seems to be acting more parasitic than mutualistic (growth was higher for E- vs. E+ plants). Clay (1990) demonstrated pathogenic relationships within plants/fungi very closely related to tall fescue, and there are some studies where this apparent physiological drain on growth appears when *N. coenophialum* was present in tall fescue (Cheplick et al. 1989; Elbersen & West 1996). As more research is done within the tall fescue-*N. coenophialum* system, the influences of various abiotic and biotic factors, as well as their interactions seem to be much more the rule than the exception.

Future Research/Directions

Results from my first study and observations in the field seem to be consistent with trends seen by Rudgers et al. (2007) – E+ stands persist as tall fescue monoculture, E- stands rapidly succeed to forest. Research nearby in Indiana (Rudgers et al. 2007, 2010) attributed the reduction in woody species in E+ plots to increased herbivore pressure by small mammals on woody stems in E+ plots (presumably avoiding consumption of E+ fescue plants). Given the lack of differences in native plant biomass and root mycorrhizal colonization between E+ and E- conditioned soil in my third experiment, and the lack of differences in tall fescue growth responses following prescribed burns in the second, it appears E+ and E- tall fescue pastures are both fundamentally capable of supporting a diverse native grassland following restoration management. In fact, the frequency at which E+ tall fescue appears in landscapes in Kentucky (Appendix 1) could be seen as a blessing for land managers, rather than a curse. High incidence of E+ tall fescue may be the reason these areas are kept open and dominated by grass species despite infrequent disturbance, as they experience

climate and conditions that would otherwise favor rapid succession to forest. High endophyte presence likely is providing a long-term reservoir of potential grassland restoration sites where we would otherwise see hardwood forest in the region.

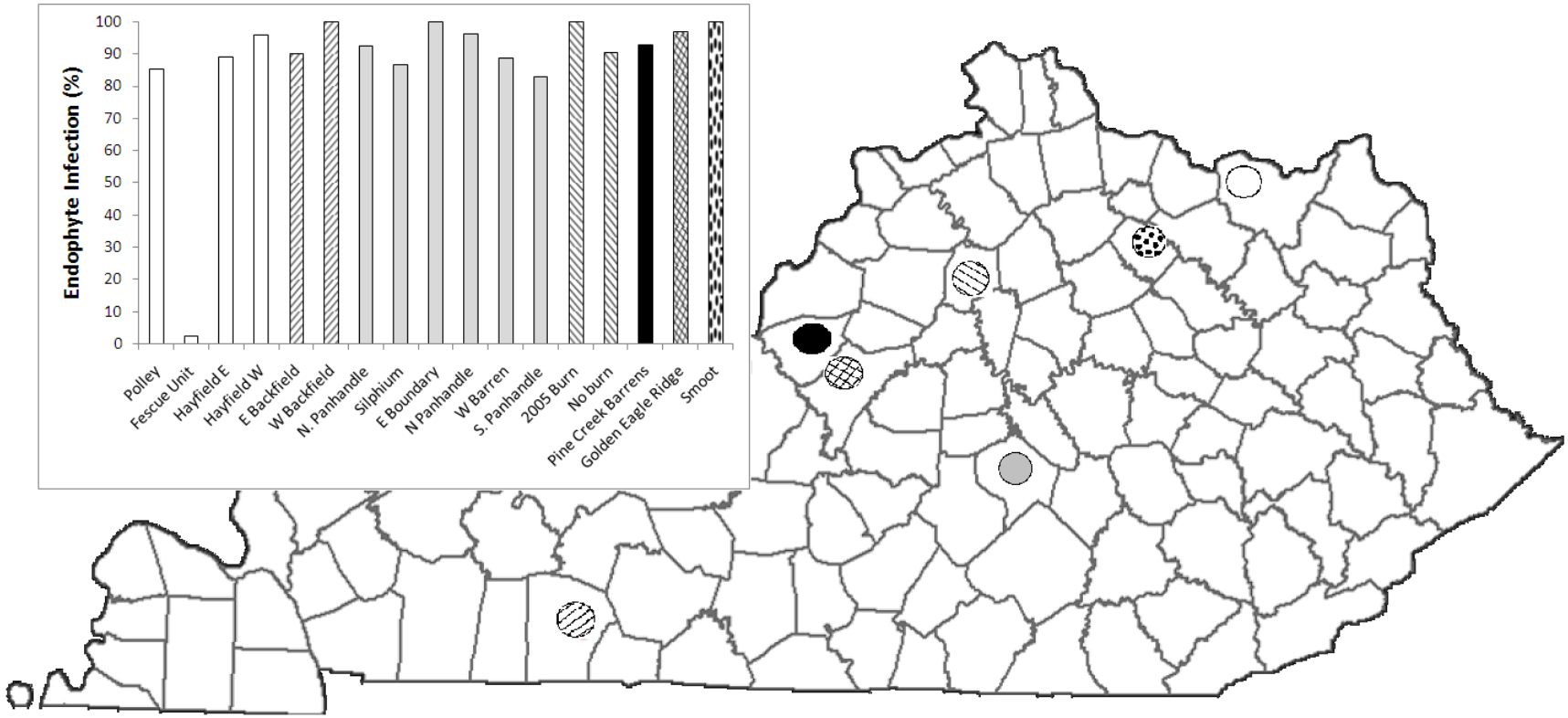
The preliminary surveys for endophyte infection frequencies within state nature preserves that I have conducted are useful, but corresponding surveys of tall fescue cover could clarify if the differences in persistence/succession between E+ and E- fields hold true across the landscape. For the seventeen fields sampled for endophyte infection frequencies (Appendix 1), I chose fields that were grass-dominated and clearly had tall fescue present. To test whether endophyte infection and tall fescue cover are correlated, areas dominated not only by grasses, but those in a successional state with woody species would also need to be included. The timeline for tall fescue's intentional spread on the landscape (1940's-1950's), as well as changes in land use marked by pasture abandonment over the past decade or so lend themselves well to such a survey. Clearly there are limits to such a survey, as knowing the exact land use history for every place surveyed might not be possible, but it would certainly provide insight into how broadly patterns in tall fescue persistence and endophyte infection impact vegetation types persisting in the landscape today.

From a restoration standpoint, research on the growth response/recovery of E+ and E- tall fescue to herbicide applications could be useful. If the majority of tall fescue pastures being targeted for restoration are highly endophyte-infected, it might be helpful to have studies verifying that E+ plants are permanently eliminated using herbicides at the label rates currently recommended. Although I did not specifically measure this, previous studies (Smith 1989; Defelice & Henning 1990) have suggested recovery of E+ plants following herbicide applications based on observations in the field. As E+ tall fescue pastures persist over longer periods of time, individual plants may

establish more robust belowground growth, possibly causing removal with herbicides to become more difficult. Herbicide resistance in response to endophyte infection has been demonstrated in Italian ryegrass (Vila-Aiub et al. 2003), and research into whether *N. coenophialum* confers herbicide resistance in tall fescue could also be useful.

The tall fescue-*N. coenophialum* symbiosis lends itself as an interesting system to study from both basic and applied viewpoints. Clearly, this fungal symbiosis can have pronounced effects on host plants under certain circumstances, but these effects may depend on a variety of biotic (ie. host plant or fungal genotype, soil microbial community) and abiotic (ie. soil fertility, texture, temperature, light levels, mowing or other management, etc.) factors. Even within the more specific symbiosis of Ky-31 tall fescue and the common toxic endophyte, which has been clearly linked with community and ecosystem scale effects (Clay & Holah 1999; Franzluebbers et al. 1999; Rudgers et al. 2004; Lemons et al. 2005; Siegrist et al. 2010), my research shows negligible differences between E+ and E- plants within the restoration context. Ky-31 with the common toxic endophyte is the most widespread association (Shelby & Dalrymple 1987) within tall fescue's eastern U.S. range, and the one most likely targeted for restoration. While there are still many interesting questions to be answered, the outlook seems promising for those wishing to remove tall fescue from abandoned pastures and establish native grassland species, regardless of endophyte status.

Appendix 1



Endophyte Infection Frequencies of seventeen different tall fescue pastures (given on the x-axis in the graph) being restored to native grassland within seven nature preserves (identified on the map with circles of fills matching the bars for pastures in each one) in Kentucky. These infection frequencies are based on collection of 20-50 tillers per pasture and should be considered preliminary.

References

Abrams MD, Hulbert LC (1987) Effect of topographic position and fire on species composition in tallgrass prairie in Northeast Kansas. American Midland Naturalist 117: 442-445.

Afkhami ME, Rudgers JA (2008) Symbiosis lost: Imperfect vertical transmission of fungal endophytes in grasses. American Naturalist 172: 405-416.

Anderson RC (2008) Growth and arbuscular mycorrhizal fungal colonization of two prairie grasses grown in soil from restorations of three ages. Restoration Ecology 16: 650-656.

Anderson RC, Hetrick BAD, Wilson GWT (1994) Mycorrhizal dependence of *Adropogon gerardii* and *Schizachyrium scoparium* in two prairie soils. American Midland Naturalist 132: 366-376.

Anderson RC, Liberta AE, Dickman LA (1984) Interaction of vascular plants and vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. Oecologia 64: 111-117.

Ansley RJ, Castellano MJ (2007) Texas wintergrass and buffalograss response to seasonal fires and clipping. Rangeland Ecology and Management 60: 154-164.

Antunes PM, Miller J, Carvalho LM, Klironomos JN, Newman JA (2008) Even after death the endophytic fungus of *Schedonorus phoenix* reduces the arbuscular mycorrhizas of other plants. Functional Ecology 22: 912-918.

Araim G, Saleem A, Arnason JT, Charest C (2009) Root colonization by an arbuscular mycorrhizal (AM) fungus increases growth and secondary metabolism of Purple Coneflower, *Echinacea purpurea* (L.) Moench. Journal of Agricultural and Food Chemistry 57: 2255-2258.

Arachevaleta M, Bacon CW, Hoveland CS, Radcliffe DE (1989) Effect of the tall fescue endophyte on plant response to environmental stress. Agronomy Journal 81: 83-90.

Arechavaleta M, Bacon CW, Plattner RD, Hoveland CS, Radcliffe DE (1992) Accumulation of ergopeptide alkaloids in symbiotic tall fescue grown under deficits of soil-water and nitrogen-fertilizer. Applied and Environmental Microbiology 58: 857-861.

Assuero SG, Matthew C, Kemp PD, Latch GCM, Barker DJ, et al. (2000) Morphological and physiological effects of water deficit and endophyte infection on contrasting tall fescue cultivars. New Zealand Journal of Agricultural Research 43: 49-61.

Assuero SG, Tognetti JA, Colabelli MR, Agnusdei MG, Petroni EC, et al. (2006) Endophyte infection accelerates morpho-physiological responses to water deficit in tall fescue. New Zealand Journal of Agricultural Research 49: 359-370.

Augustine DJ, Milchunas DG (2009) Vegetation responses to prescribed burning of grazed shortgrass steppe. Rangeland Ecology and Management 62: 89-97.

Bacon CW (1995) Toxic endophyte-infected tall fescue and range grasses: Historical perspectives. Journal of Animal Science 73: 861-870.

Bacon CW, Siegel MR (1988) Endophyte parasitism of tall fescue. Journal of Production Agriculture 1: 45-55.

Ball D, Lacefield G, Hoveland CS (1991) The tall fescue endophyte. UK Extension Publication AGR-149. URL http://www.ca.uky.edu/agc/pubs/agr/agr149/agr149.htm [accessed 17 Jun 2010]

Ball DM, Pedersen JF, Lacefield GD (1993) The tall-fescue endophyte: Evolution meets economic in the tale of the nation's most popular planted grass, which owes many of its qualities to a fungus toxic to livestock. American Scientist 81: 370-380.

Bardgett RD, Leemans DK, Cook P, Hobbs PJ (1997) Seasonality of soil biota of grazed and ungrazed hill grasslands. Soil Biology and Biochemistry 29: 1285-1294.

Barnes TG (2004) Strategies to convert exotic grass pastures to tall grass prairie communities. Weed Technology 18: 1364-1370.

Barnes TG (2007) Using herbicides to rehabilitate native grasslands. Natural Areas Journal 27: 56-65.

Barnes TG, Madison LA, Sole JD, Lacki MJ (1995) An assessment of habitat quality for northern bobwhite quail in tall fescue dominated fields. Wildlife Society Bulletin 23: 231-237.

Baskin CC, Baskin JM (1975) The cedar glade flora of Bullitt County, Kentucky. Castanea 40: 184-190.

Baskin CC, Baskin JM (1998) Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego, CA: Academic Press.

Baskin JM, Baskin CC, Chester EW (1994) The big barrens region of Kentucky and Tennessee: further observations and considerations. Castanea 59(3): 226-254.

Baskin JM, Baskin CC, Chester EW (1999) The big barrens region of Kentucky and Tennessee. In: Anderson RC, Fralish JS, Baskin JM, editors. Savannas, Barrens, and Rock

Outcrop Plant Communities of North America. New York, NY: Cambridge University Press. pp. 190-205.

Bayat F, Mirlohi A, Khodambashi M (2009) Effects of endophytic fungi on some drought tolerance mechanisms of tall fescue in hydroponics culture. Russian Journal of Plant Physiology 56: 510-516.

Baylis GTS (1974) The evolutionary significance of phycomycetous mycorrhizas. In: Mechanisms of Regulation of Plant Growth. R. Soc. N.Z. Bull. No. 12. pp. 191-193.

Belesky DP, Burner DM, Ruckle JM (2008) Does endophyte influence resource acquisition and allocation in defoliated tall fescue as a function of microsite conditions? Environmental and Experimental Botany 63: 368-377.

Belesky DP, Fedders JM (1995) Tall fescue development in response to *Acremonium coenophialum* and soil acidity. Crop Science 35: 529-533.

Belesky DP, Stuedemann JA, Plattner RD, Wilkinson SR (1988) Ergopeptine alkaloids in grazed tall fescue. Agronomy Journal 80: 209-212.

Bennett AE, Bever JD (2007) Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology 88: 210-218.

Bernard EC, Gwinn KD, Pless CD, Williver CD (1997) Soil invertebrate species diversity and abundance in endophyte-infected tall fescue pastures. In: Bacon CW, Hill NS, editors. *Neotyphodium*/Grass Interactions. New York, NY: Plenum Press. pp. 125-135.

Bingham MA, Biondini M (2009) Mycorrhizal hyphal length as a function of plant community richness and composition in restored northern tallgrass prairie (USA). Rangeland Ecology and Management 62: 60-67.

Blumenthal DM (2005) Interrelated causes of plant invasion. Science 310: 243-244.

Blumenthal DM (2006) Interactions between resource availability and enemy release in plant invasion. Ecology Letters 9: 887-895.

Blumenthal D, Jordan NR, Svenson EL (2005) Effects of prairie restoration on weed invasions. Agriculture, Ecosystems and Environment 107: 221-230.

Bouton JH, Gates RN, Belesky DP, Owsley M (1993) Yield and persistence of tall fescue in southeastern coastal plain after removal of its endophyte. Agronomy Journal 85: 52-55.

Bouton JH, Gates RN, Hoveland CS (2001) Selection for persistence in endophyte-free Kentucky 31 tall fescue. Crop Science 41: 1026-1028.

Bradley K, Drijber RA, Knops J (2006) Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 38: 1583-1595.

Brejda JJ, Moser LE, Vogel KP (1998) Evaluation of switchgrass rhizosphere microflora for enhancing seedling yield and nutrient uptake. Agronomy Journal 90: 753-758.

Brejda JJ, Yocom DH, Moser LE, Waller SS (1993) Dependence of 3 Nebraska sandhills warm-season grasses on vesicular-arbuscular mycorrhizae. Journal of Range Management 46: 14-20.

Brodie E, Edwards S, Clipson N (2002) Bacterial community dynamics across a floristic gradient in a temperate upland grassland ecosystem. Microbial Ecology 44: 260-270.

Brosi, GB (2011) The response of tall fescue and its fungal endophyte to climate change. M.S. Thesis. University of Kentucky (Lexington). URL http://uknowledge.uky.edu/gradschool_theses/126 [accessed 25 Jun 2011]

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytologist 154: 275-304.

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320: 37-77.

Buck GW, West CP, Elbersen HW (1997) Endophyte effect on drought tolerance in diverse *Festuca* species. In: Bacon CW, Hill NS, editors. Neotyphodium/Grass Interactions. New York, NY: Plenum Press. pp. 141-143.

Bultman TL, Bell GD (2003) Interaction between fungal endophytes and environmental stressors influences plant resistance to insects. Oikos 103: 182-190.

Burrows RL, Pfleger FL (2002) Host responses to AMF from plots differing in plant diversity. Plant and Soil 240: 169-179.

Buyer JS, Zuberer DA, Nichols KA, Franzluebbers AJ (2011) Soil microbial community function, structure, and glomalin in response to tall fescue endophyte infection. Plant and Soil 339: 401-412.

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427: 731-733.

Canals RM, Emeterio LS, Oreja A (2008) Chances of loss of fungal endophytes in agronomic grasses: A case study for *Lolium rigidum*. Agriculture , Ecosystems and Environment 127: 146-152.

Cavender N, Knee M (2006) Relationship of seed source and arbuscular mycorrhizal fungi inoculum type to growth and colonization of big bluestem (*Andropogon gerardii*). Plant and Soil 28: 57-65.

Chagnon SA, Kunkel KE, Winstanley D (2002) Climate factors that caused the unique tall grass prairie in the central United States. Physical Geography 23: 259-280.

Cheplick GP, Clay K, Marks S (1989) Interactions between infection by endophytic fungi and nutrient limitation in the grasses *Lolium perenne* and *Festuca arundinacea*. New Phytologist 111: 89-97.

Chester EW, Wofford BE, Baskin JM, Baskin CC (1997) A floristic study of barrens on the Southewestern Pennyroyal Plain, Kentucky and Tennessee. Castanea 62: 161-172.

Chu-Chou M, Guo B, An ZQ, Hendrix JW, Ferriss RS, et al. (1992) Suppression of mycorrhizal fungi in fescue by the *Acremonium coenophialum* endophyte. Soil Biology and Biochemistry 24: 633-637.

Clark RB (2002) Differences among mycorrhizal fungi for mineral uptake per root length of switchgrass grown in acidic soil. Journal of Plant Nutrition 25: 1753-1772.

Clark RB, Zeto SK, Zobel RW (1999) Arbuscular mycorrhizal fungal isolate effectiveness on growth and root colonization of *Panicum virgatum* in acidic soil. Soil Biology and Biochemistry 31: 1757-1763.

Clay K (1990) Comparative demography of three graminoids infected by systemic, clavicipitaceous fungi. Ecology 71: 558-570.

Clay K, Hardy TN, Hammond Jr. AM (1985) Fungal endophytes of grasses and their effects on an insect herbivore. Oecologia 66: 1-5.

Clay K, Holah J (1999) Fungal endophyte symbiosis and plant diversity in successional fields. Science 285: 1742-1744.

Clay K, Marks S, Cheplick GP (1993) Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. Ecology 74: 1767-1777.

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. American Naturalist 160: S99-S127.

Colwell RK (2006) EstimateS: Statistical estimation of species richness and shared species from samples. Version 8. Persistent URL <purl.oclc.org/estimates>

Cuomo GJ, Anderson BE, Young LJ (1998) Harvest frequency and burning effects on vigor of native grasses. Journal of Range Management 51: 32-36.

D'Angelo DM, Karathanasis AD, Sparks EJ, Ritchey SA, Wehr-McChesney SA (2005) Soil carbon and microbial communities at mitigated and late successional bottomland forest wetlands. Wetlands 25: 162-175.

Davidson AD, Ponce E, Lightfoot DC, Fredrickson EL, Brown JH, et al. (2010) Rapid response of a grassland ecosystem to an experimental manipulation of a keystone rodent and domestic livestock. Ecology 91: 3189-3200.

Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: A general theory of invasibility. Journal of Ecology 88: 528-534.

Defelice MS, Henning JC (1990) Renovation of endophyte (*Acremonium coenophialum*)-infected tall fescue (*Festuca arundinacea*) pastures with herbicides. Weed Science 38: 628-633.

DeSelm HR (1994) Tennessee barrens. Castanea 59: 214-225.

Dhillion SS (1992) Evidence for host-mycorrhizal preference in native grassland species. Mycological Research 96: 359-362.

Dhillion SS, Anderson RC (1993) Growth dynamics and associated mycorrhizal fungi of little bluestem grass [*Schizachyrium scoparium* (Michx.) Nash] on burned and unburned sand prairies. New Phytologist 123: 77-91.

Dicken SN (1935) Kentucky karst landscapes. The Journal of Geology 43: 708-728.

DiTomaso JM, Brooks ML, Allen EB, Minnich R, Rice PM, et al. (2006) Control of invasive weeds with prescribed burning. Weed Technology 20: 535-548.

Duncan RS, Anderson CB, Sellers HN, Robbins EE (2008) The effect of fire reintroduction on endemic and rare plants of a Southeastern glade ecosystem. Restoration Ecology 16(1): 39-49.

Elbersen HW, West CP (1996) Growth and water relations of field-grown tall fescue as influenced by drought and endophyte. Grass and Forage Science 51: 333-342.

Elton CS (1958) The Ecology of Invasions by Animals and Plants. London: Methuen.

Eom A-H, Hartnett DC, Wilson GWT, Figge DAH (1999) The effect of fire, mowing, and fertilizer amendment on arbuscular mycorrhizas in the tallgrass prairie. American Midland Naturalist 142: 55-70.

Faeth SH, Bultman TL (2002) Endophytic fungi and interactions among host plants, herbivores, and natural enemies. In: Tscharntke T, Hawkins BA, editors. Multitrophic Level Interactions. Cambridge: Cambridge University Press. pp. 89-123.

Faeth SH, Fagan WF (2002) Fungal endophytes: Common host plant symbionts but uncommon mutualists. Integrative and Comparative Biology 42: 360-368.

Faeth SH, Sullivan TJ (2003) Mutualistic asexual endophytes in a native grass are usually parasitic. American Naturalist 161: 310-325.

Fisher RJ, Davis SK (2011) Post-fledging dispersal, habitat use, and survival of Sprague's pipits: Are planted grasslands a good substitute for native? Biological Conservation 144: 263-271.

Foster BL, Kindscher K, Houseman GR, Murphy CA (2009) Effects of hay management and native species sowing on grassland community structure, biomass, and restoration. Ecological Applications 19: 1884-1896.

Franzluebbers AJ (2006) Short-term responses on soil C and N fractions to tall fescue endophyte infection. Plant and Soil 282: 153-164.

Franzluebbers AJ, Nazih N, Steudemann JA, Fuhrmann JJ, Schomberg HH, et al. (1999) Soil carbon and nitrogen pools under low- and high-endophyte infected tall fescue. Soil Science Society of America Journal 63: 1687-1694.

Franzluebbers AJ, Stuedemann JA (2005) Soil carbon and nitrogen pools in response to tall fescue endophyte infection, fertilization, and cultivar. Soil Science Society of America Journal 69: 396-403.

Fuhlendorf SD, Townsend DE, Elmore RD, Engle DM (2010) Pyric-herbivory to promote rangeland heterogeneity: Evidence from small mammal communities. Rangeland Ecology and Management 63: 670-678.

Gai JP, Christie P, Cai XB, Fan JQ, Zhang JL, et al. (2009) Occurrence and distribution of arbuscular mycorrhizal fungal species in three types of grassland community of the Tibetan Plateau. Ecological Research 24: 1345-1350.

Ghimire SR, Charlton ND, Craven KD (2009) the mycorrhizal fungus, *Sebacina vermifera*, enhances seed germination and biomass production in switchgrass (*Panicum virgatum* L). Bioenergy Research 2: 51-58.

Gillespie IG, Allen EB (2004) Fire and competition in a southern California grassland: Impacts on the rare forb *Erodium macrophyllum*. Journal of Applied Ecology 41: 643-652.

Gleason HA (1922) The vegetational history of the Middle West. Annals of the Association of American Geographers 12: 39-85.

Global Restoration Network (GRN). (Undated) Grassland. URL http://www.globalrestorationnetwork.org/ecosystems/grassland/ [accessed 22 May 2011]

Grant WE, Birney EC (1979) Small mammal community structure in North American grasslands. Journal of Mammalogy 60: 23-36.

Grayston SJ, Campbell CD, Bardgett RD, Mawdsley JL, Clegg CD, et al. (2004) Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Applied Soil Ecology 25: 63-84.

Gundel PE, Garibaldi LA, Tognetti PM, Aragon R, Ghersa CM, et al. (2009) Imperfect vertical transmission of the endophyte *Neotyphodium* in exotic grasses in grasslands of the flooding Pampa. Microbial Ecology 57: 740-748.

Guo BZ, Hendrix JW, An Z-Q, Ferriss RS (1992) Role of *Acremonium* endophyte of fescue on inhibition of colonization and reproduction of mycorrhizal fungi. Mycologia 84: 882-885.

Gustafson DJ, Casper BB (2004) Nutrient addition affects AM fungal performance and expression of plant/fungal feedback in three serpentine grasses. Plant and Soil 259: 9-12.

Gwinn KD, Fribourg HA, Waller JC, Saxton AM, Smith MC (1998) Changes in *Neotyphodium coenophialum* infestation levels in tall fescue pastures due to different grazing pressures. Crop Science 38: 201-204.

Hall SL (2010) Tall fescue and eastern red-cedar alter fire intensity in grassland. The Lady-Slipper 25:4-5. URL http://www.knps.org/newsletters/The%20Lady%20Slipper%20Spring%202010%20v25%20n1.pdf [accessed 21 Jun 2011]

Hall SL, Barney RJ (2010) A quantitative method for assigning abundance classifications to leaf beetles (Coleoptera: Chrysomelidae) in Kentucky. Natural Areas Journal 31: 95-105.

Hannaway D, Fransen S, Cropper J, Teel M, Chaney M, et al. (1999) Tall Fescue. Oregon State University Extension Publication PNW 504. URL http://extension.oregonstate.edu/catalog/html/pnw/pnw504/ [accessed 14 Mar 2009]

Harley JR, Smith SE (1983) Mycorrhizal Symbiosis. New York,NY: Academic Press.

Harper CA, Bates GE, Gudlin MJ, Hansbrough MP (2004) A Landowner's guide to native warm-season grasses in the mid-South. UT Extension Publication PB 1746. URL http://www.wildlifemanagement.info/files/nwsg_9.pdf [accessed 2 Sept 2009]

Hartnett DC, Samenus RJ, Fischer LE (1994) Plant demographic resposes to mycorrhizal symbiosis in tallgrass prairie. Oecologia 99: 21-26.

Hartnett DC, Wilson GWT (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80: 1187-1195.

Henson J, Safley M (2009) NRCS Plant Information and Conservation Practice Standards. In: Fribourg HA, Hannaway DB, West CP, editors. Tall Fescue for the Twenty-first Century. Madison, WI: American Society of Agronomy. pp. 483-487

Hetrick BAD, Wilson GWT, Todd TC (1990) Differential responses of C3 and C4 grasses to mycorrhizal symbiosis, phosphorus fertilization, and soil microorganisms. Canadian Journal of Botany 68: 461-467.

Hill NS, Belesky DP, Stringer WC (1991) Competitiveness of tall fescue as influenced by *Acremonium coenophialum*. Crop Science 31: 185-190.

Hill NS, Belesky DP, Stringer WC (1998) Encroachment of endophyte-infected on endophyte-free tall fescue. Annals of Botany 81: 483-488.

Hill NS, Bouton JH, Hiatt III EE, Bittle B (2005) Seed maturity, germination, and endophyte relationships in tall fescue. Crop Science 45: 859-863.

Hill NS, Pachon JG, Bacon CW (1996) *Acremonium coenophialum*-mediated short- and long-term drought acclimation in tall fescue. Crop Science 36: 665-672

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, et al. (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13: 394-407.

Holah JC, Alexander HM (1999) Soil pathogenic fungi have the potential to affect the co-existence of two tallgrass prairie species. Journal of Ecology 87: 598-608.

Hopkins AA, Alison MW (2006) Stand persistence and animal performance for tall fescue endophyte combinations in the South Central USA. Agronomy Journal 98: 1221-1226.

Huag I, Wubet T, Weiss M, Aguirre N, Weber M, et al. (2010) Species-rich but distinct arbuscular mycorrhizal communities in reforestation plots on degraded pastures and in neighboring pristine tropical mountain rain forest. Tropical Ecology 51: 125-148.

International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM). (Undated) Recipes for voucher preservation. URL http://invam.caf.wvu.edu/methods/recipes.htm [accessed 28 Jun 2011]

Jakobsen I, Anderson AJ (1982) Vesicular-arbuscular mycorrhiza and growth in barley: Effects of irradiation and heating of soil. Soil Biology and Biochemistry 14: 171-178.

Jenkins M, Franzluebbers A, Humayoun S (2006) Assessing short-term responses of prokaryotic communities in bulk and rhizosphere soils to tall fescue endophyte infection. Plant and Soil 289: 309-320.

Jenkins SE, Jenkins MA (2006) Effects of prescribed fire on the vegetation of a savanna-glade complex in northern Arkansas. Southeastern Naturalist 5: 113-126.

Johnson NC (1998) Responses of *Salsola kali* and *Panicum virgatum* to mycorrhizal fungi, phosphorus and soil organic matter: implications for reclamation. Journal of Applied Ecology 35: 86-94.

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. PNAS 107: 2093-2098.

Jones RN (2005) Plant Life of Kentucky. Lexington: University Press of Kentucky.

Jones MD, Smith SE (2004) Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? Canadian Journal of Botany 82: 1089-1109.

Jones-Farrand DT, Johnson DH, Burger LW Jr., Ryan MR (2007) Grassland establishment for wildlife conservation. In: Haufler JB, editor. Fish and Wildlife Response to Farm Bill Conservation Practices. Bethesda, MD: The Wildlife Society.

Ju HJ, Hill NS, Abbott T, Ingram KT (2006) Temperature influences on endophyte growth in tall fescue. Crop Science 46: 404-412.

Kaye TN, Pendergrass KL, Finley K, Boone Kauffman J (2001) The effect of fire on the population viability of an endangered prairie plant. Ecological Applications 11: 1366-1380.

Kelrick MI, Kasper NA, Bultman TL, Taylor S (1990) Direct interactions between infected and un-infected individuals of *Festuca arundinacea*: differential allocation to shoot and root biomass. Proceedings of the International Symposium *Acremonium*/Grass Interactions: 21-29.

Kentucky Exotic Pest Plant Council (KY-EPPC). 2008. Exotic Plant List- Severe Threat. URL http://www.se-eppc.org/ky/list.htm [accessed 29 Jun 2011]

Kindscher K, Tieszen LL (1998) Floristic and soil organic matter changes after five and thirty-five years of native tallgrass prairie restoration. Restoration Ecology 6: 181-196.

Kirkby KA, Pratley JE, Hume DE, Faville MJ, An M, et al. (2011) Incidence of endophyte *Neotyphodium occultans* in *Lolium rigidum* from Australia. Weed Research 51: 261-272.

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67-70.

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84: 2292-2301.

Klironomos J, Zobel M, Tibbett M, Stock WD, Rillig MC, et al. (2011) Forces that structure plant communities: quantifying the importance of the mycorrhizal symbiosis. New Phytologist 89: 366-370.

Koide RT (1985) The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection. New Phytologist 99: 449-462.

Koide RT, Goff MD, Dickie IA (2000) Component growth efficiencies of mycorrhizal and nonmycorrhizal plants. New Phytologist 148: 163-168.

Koide RT, Kabir Z (2000) Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. New Phytologist 148: 511-517.

Koslowsky SD, Boerner REJ (1989) Interactive effects of aluminum, phosphorus and mycorrhizae on growth and nutrient uptake of *Panicum virgatum* L. (Poaceae). Environmental Pollution 61: 107-125.

Kotanen PM (2004) Revegetation following soil disturbance and invasion in a California meadow: a 10-year history of recovery. Biological Invasions 6: 245-254.

Küchler AW (1964) Potential natural vegetation of the conterminous United States. American Geographical Society, Special Publication No 36.

Kutschbach-Brohl L, Washburn BE, Bernhardt GE, Chipman RB, Francoeur LC (2010) Arthropods of a semi-natural grassland in an urban environment: The John F. Kennedy international airport, New York. Journal of Insect Conservation 14: 347-358.

Lacefield GD, Henning JC (1986) Alternatives for fungus infected tall fescue. UK Agricultural Experiment State Report AGR-119. URL http://www.ca.uky.edu/agc/pubs/agr/agr119/agr119.htm [accessed 17 Jun 2010]

Lacefield GD, Henning JC, Phillips TD (2003) Tall fescue. University of Kentucky Agricultural Experiment Station Report AGR-59. URL http://www.ca.uky.edu/agc/pubs/agr/agr59/agr59.pdf [accessed 9 Jun 2011]

Land Between the Lakes (LBL). Undated. Oak-Grassland Demonstration Restoration Area. URL http://www.lbl.org/NRMOakGrassland.html [accessed 29 Jun 2011]

Landis FC, Gargas A, Givnish TJ (2005) The influence of arbuscular mycorrhizae and light on Wisconsin (USA) sand savanna understories. Mycorrhiza 15: 547-553.

Lata H, Andrade ZD, Schaneberg B, Bedir E, Khan I, et al. (2003) Arbuscular mycorrhizal inoculation enhances survival rates and growth of micropropogated plantlets of *Echinacea pallida*. Planta Medica 69: 679-682.

Latch GCM (1993) Physiological interactions of endophytic fungi and their hosts. Biotic stress tolerance imparted to grasses by endophytes. Agriculture, Ecosystems and Environment 44: 143-156.

Lawless PJ, Baskin JM, Baskin CC (2004) The floristic ecology of xeric limestone prairies in Kentucky, and a comparison to limestone cedar and deep-soil barrens. SIDA 21: 1055-1079.

Lawless PJ, Baskin JM, Baskin CC (2006) Xeric limestone prairies of Eastern United States: Review and synthesis. The Botanical Review 72: 235-272.

Lekberg Y, Hammer EC, Olsson PA (2010) Plants as resource islands and storage units - adopting the mycocentric view of arbuscular mycorrhizal networks. Fems Microbiology Ecology 74: 336-345.

Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytologist 168: 189-204.

Lemons A, Clay K, Rudgers JA (2005) Connecting plant-microbial interactions above and belowground: A fungal endophyte affects decomposition. Oecologia 145: 595-604.

Li LF, Li T, Zhao ZW (2007) Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land, an old field, and a never-cultivated field in a hot and arid ecosystem of southwest China. Mycorrhiza 17: 655-665.

Limb RF, Engle DM, Alford AL, Hellgren EC (2010) Tallgrass prairie plant community dynamics along a canopy cover gradient of eastern redcedar (*Juniperus virginiana*). Rangeland Ecology and Management 63: 638-644.

Lindstrom MJ, Shumacher TE, Blecha ML (1994) Management considerations for returning CRP lands to crop production. Journal of Soil and Water Conservation 49: 420-425.

Liu Q, Parsons AJ, Xue H, Fraser K, Ryan GD, et al. (2011) Competition between foliar *Neotyphodium lolii* endophytes and mycorrhizal *Glomus* spp. fungi in *Lolium perenne* depends on resource supply and host carbohydrate content. Functional Ecology: unknown (Early View).

Lyons PC, Plattner RD, Bacon CW (1986) Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science 232: 487-489.

Madison LA, Barnes TG, Sole JD (2001) Effectiveness of fire, disking, and herbicide to renovate tall fescue fields to northern bobwhite habitat. Wildlife Society Bulletin 29: 706-712.

Mack KML, Rudgers JA (2008) Balancing multiple mutualists: Asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. Oikos 117: 310-320.

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal communities assembly and ecosystem functioning. Science 316: 1746-1748.

Malinowski DP, Belesky DP, Balingar VC, Fedders JM (1997) Involvement of *Neotyphodium coenophialum* in phosphorous uptake by tall fescue (*Festuca arundiancea* Schreb.). In: Bacon CW, Hill NS, editors. Neotyphodium/Grass Interactions. New York, NY: Plenum Press. pp. 183-185.

Malinowski DP, Belesky DP, Fedders JM (1999) Endophyte infection may affect the competitive ability of tall fescue grown with red clover. Journal of Agronomy and Crop Science 183: 91-101.

Marks S, Clay K (1996) Physiological responses of *Festuca arundinacea* to fungal endophyte infection. New Phytologist 133: 727-733.

Marks S, Clay K, Cheplick GP (1991) Effects of fungal endophytes on interspecific and intraspecific competition in the grasses *Festuca arundinacea* and *Lolium perenne*. Journal of Applied Ecology 28: 194-204.

Masters RA, Vogel KP, Mitchell RB (1992) Response of Central Plains tallgrass prairies to fire, fertilizer, and atrazine. Journal of Range Management 45: 291-295.

McCulley RL, Burke IC, Nelson JA, Lauenroth WK, Knapp AK, et al. (2005) Regional patterns in carbon cycling across the Great Plains of North America. Ecosystems 8: 106-121.

McCune B, Mefford MJ (1999) PC-ORD. Multivariate Analysis of Ecological Data. Version 4.41 MjM Software, Gleneden Beach, Oregon, U.S.A.

McEwan RW, McCarthy BC (2008) Anthropogenic disturbance and the formation of oak savanna in central Kentucky, USA. Journal of Biogeography 35: 965-975.

McGonigle TP (1988) A numerical analysis of published field trials with vesicular-arbuscular mycorrhizal fungi. Functional Ecology 2: 473-478.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115: 495-501.

Meredith JA, Anderson RC (1992) The influence of varied microbial substrate conditions on the growth and mycorrhizal colonization of little bluestem [*Schizachyrium scoparium* (Michx.) Nash]. New Phytologist 121: 235-242.

Miller RM (1987) The ecology of vesicular-arbuscular mycorrhizae in grass- and shrublands. In: Safir GR, editor. Ecophysiology of VA Mycorrhizal Plants. Boca Raton, FL: CRC Press. pp. 135-170.

Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral pathogens. Nature 421: 625-627.

Moyes AB, Witter MS, Gamon JA (2005) Restoration of native perennials in a California annual grassland after prescribed spring burning and solarization. Restoration Ecology 13: 659-666.

Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288: 81-90.

Newman JA, Abner ML, Dado RG, Gibson DJ, Brookings A, et al. (2003) Effects of elevated CO2, nitrogen and fungal endophyte-infection on tall fescue: Growth, photosynthesis, chemical composition and digestibility. Global Change Biology 9: 425-437.

Nüsslein K, Tiedje JM (1999) Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Applied and Environmental Microbiology 65: 3622-3626.

Orr SP, Rudgers JA, Clay K (2005) Invasive plants can inhibit native tree seedlings: Testing potential allelopathic mechanisms. Plant Ecology 181: 153-165.

Pecetti L, Romani M, Annicchiarico P, Piano E (2008) Tall fescue adaptation to low nitrogen fertilization in relation to germplasm type and endophyte infection. Journal of Agronomy and Crop Science 194: 244-247.

Pierce AM, Reich PB (2010) The effects of eastern red cedar (*Juniperus virginiana*) invasion and removal on a dry bluff prairie ecosystem. Biological Invasions 12: 241-252.

Piotrowski JS, Lekberg Y, Harner MJ, Ramsey PW, Rillig MC (2008) Dynamics of mycorrhizae during development of riparian forests along an unregulated river. Ecography 31: 245-253.

Pringle A, Bever JD (2008) Analogous effects of arbuscular mycorrhizal fungi in the laboratory and a North Carolina field. New Phytologist 180: 162-175.

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, et al. (2009) Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution, and Systematics 40: 699-715.

Pyke DA, Brooks ML, D'Antonio C (2010) Fire as a restoration tool: A decision framework for predicting the control or enhancement of plants using fire. Restoration Ecology 18: 274-284.

Rahman MH, Saiga S (2005) Endophytic fungi (*Neotyphodium coenophialum*) affect the growth and mineral uptake, transport, and efficiency ratios in tall fescue (*Festuca arunidinacea*). Plant and Soil 272: 163-171.

Raunkiaer C (1934) The life forms of plants and statistical plant geography; being the collected papers of C. Raunkiaer; Translated by Gilbert-Carter AIF, and edited by Tansley AG. Oxford, United Kingdon: The Clarendon Press.

Rhazi M, Grillas P, Rhazi L, Charpentier A, Medail F (2009) Competition in microcosm between a clonal plant species (*Bolboschoenus maritimus*) and a rare quillwort (*Isoetes setacea*) from Mediterranean temporary pools of southern France. Hydrobiologia 634: 115-124.

Rhoades C, Barnes T, Washburn B (2002) Prescribed fire and herbicide effects on soil processes during barrens restoration. Restoration Ecology 10: 656-664.

Rhoades CC, Miller SP, Skinner DL (2005) Forest vegetation and soil patterns across glade-forest ecotones in the knobs region of northeastern Kentucky, USA. The American Midland Naturalist 154: 1-10.

Rhoades CC, Shea MM (2003) Forest type influences response of vegetation and soil chemistry to edge clearing in dry glades (Kentucky). Ecological Restoration 21: 42-43.

Rhodes MT, Peterson JA, Kerley MS, Garner HE, Laughlin MH (1991) Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. Journal of Animal Science 69: 2033-2043.

Rice PM, Toney CJ, Bedunah DJ, Carlson CE (1997) Plant community diversity and growth form responses to herbicide applications for control of *Centaurea maculosa*. Journal of Applied Ecology 34:1397-1412.

Richardson ML (2010) Effects of grassland succession on communities of small mammals in Illinois, USA. Biologia 65: 344-348.

Rinella MJ, Maxwell BD, Fay PK, Weaver T, Sheley RL (2009) Control effort exacerbates invasive-species problem. Ecological Applications 19: 155-162.

Roberts CA, Spiers DE, Karr AL, Benedict HR, Sleper DA, et al. (2002) Use of a rat model to evaluate tall fescue seed infected iwth introduced strains of *Neotyphodium coenophialum*. Journal of Agricultural and Food Chemistry 50: 5742-5745.

Rodriguez RJ, White JF Jr., Arnold AE, Redman RS (2009) Fungal endophytes: Diversity and functional roles. New Phytologist 182: 314-330.

Rudgers JA, Clay K (2007) Endohpyte symbiosis with tall fescue: How strong are the impacts on communities and ecosystems? Fungal Biology Reviews 21: 107-124.

Rudgers JA, Clay K (2008) An invasive plant-fungal mutualism reduces arthropod diversity. Ecology Letters 11: 831-840.

Rudgers JA, Fischer S, Clay K (2010) Managing plant symbiosis: Fungal endophyte genotype alters plant community composition. Journal of Applied Ecology 47: 468-477.

Rudgers JA, Holah J, Orr SP, Clay K (2007) Forest succession suppressed by an introduced plant-fungal symbiosis. Ecology 88: 18-25.

Rudgers JA, Koslow JM, Clay K (2004) Endophytic fungi alter relationships between diversity and ecosystem properties. Ecology Letters 7: 42-51.

Rudgers JA, Mattingly WB, Koslow JM (2005) Mutualistic fungus promotes plant invasion into diverse communities. Oecologia 144: 463-471.

Rudgers JA, Orr S (2009) Non-native grass alters growth of native tree species via leaf and soil microbes. Journal of Ecology 97: 247-255.

Ruffner ME, Barnes TG (2004) The efficacy of herbicides for eradicating tall fescue. Proceedings of the North American Prairie Conference 19: 67-74.

Ruffner ME, Barnes TG (2010) Natural grassland response to herbicides and application timing for selective control of tall fescue, an invasive cool-season grass. Invasive Plant Science and Management 3: 219-228.

Ruffner CM, Groininger JW (2006) Making the case for fire in Southern Illinois forests. Journal of Forestry: 78-83.

Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: A continuum of interactions with host plants. Annual Review of Ecology and Systematics 29: 319.

Salminen SO, Richmond DS, Grewal SK, Grewal PS (2005) Influence of temperature on alkaloid levels and fall armyworm performance in endophytic tall fescue and perennial ryegrass. Entomologia Experimentalis Et Applicata 115: 417-426.

Samson F, Knopf F (1994) Prairie conservation in North America. Bioscience 44: 418-421.

Samson FB, Knopf FL, Ostile WR (1998) Grasslands. In Status and Trends of the Nation's Biological Resources, vol. 2. U.S. Department of Interior, US Geological Survey. pp. 437-472. URL http://www.nwrc.usgs.gov/sandt/Grasslnd.pdf [accessed 4 Feb 2010]

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Annual Review of Plant Biology 55: 315-340.

Schmidt IK, Michelsen A, Jonasson S (1997) Effects of labile soil carbon on nutrient partitioning between an arctic graminoid and microbes. Oecologia 112: 557-565.

Schüssler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: Phylogeny and evolution. Mycological Research 105: 1413-1421.

Seeling B, Jungk A (1996) Utilization of organic phosphorus in calcium chloride extracts of soil by barley plants and hydrolysis by acid and alkaline phosphatases. Plant and Soil 178: 179-184

Shelby RA, Dalrymple LW (1987) Incidence and distribution of the tall fescue endophyte in the United States. Plant Disease 71: 783-786.

Shelby RA, Dalrymple LW (1993) Long-term changes of endophyte infection in tall fescue stands. Grass and Forage Science 48: 356-361.

Sheley RL, Mangold JM, Anderson JL (2006) Potential for successional theory to guide restoration of invasive-plant-dominated rangeland. Ecological Monographs 76: 365-379.

Siegel MR, Bush LP (1994) Importance of endophytes in forage grasses, a statement of problems and selection of endophytes. In: Bacon CW, White JF, Jr., editors. Biotechnology of Endophytic Fungi of Grasses. Boca Raton, FL: CRC Press. pp. 135-150.

Siegel MR, Latch GCM, Bush LP, Fannin FF, Rowan DD, et al. (1990) Fungal endophyte-infected grasses: Alkaloid accumulation and aphid response. Journal of Chemical Ecology 16: 1573-1561.

Siegrist JA (2008) The endophyte effect: Ecosystem consequences of the tall fescue-fungal endophyte symbiosis. M.S. Thesis. University of Kentucky (Lexington).

Siegrist JA, McCulley RL, Bush LP, Phillips TD (2010) Alkaloids may not be responsible for endophyte-associated reductions in tall fescue decomposition rates. Functional Ecology 24: 460-468.

Smith AE (1989) Herbicides for killing tall fescue (*Festuca arundinacea*) infected with fescue endophyte (*Acremonium coenophialum*). Weed Technology 3: 485-489.

Smith SE, Read DJ (1997) Mycorrhizal Symbiosis. San Diego, CA: Academic Press.

Society for Ecological Restoration (International Science & Policy Working Group) (2004) The SER International Primer on Ecological Restoration. Tucson, AZ: Society for Ecological Restoration International. URL http://www.ser.org/pdf/primer3.pdf [accessed 8 Jun 2011]

Spyreas G, Gibson DJ, Middleton BA (2001) Effects of endophyte infection in tall fescue (*Festuca arundinacea*: Poaceae) on community diversity. International Journal of Plant Sciences 162: 1237-1245.

Stanley TR (2010) Land use and small mammal predation effects on shortgrass prairie birds. Journal of Wildlife Management 74: 1825-1833.

Taft JB (2003) Fire effects on community structure, composition, and diversity in a dry sandstone barrens. Journal of the Torrey Botanical Society 130(3): 170-192.

Talbot JM, Allison SD, Treseder KK (2008) Decomposers in disguise: Mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Functional Ecology 22: 955-963.

Thorpe AS, Thelen GC, Diaconu A, Callaway RM (2009) Root exudate is allelopathic in invaded community but not in native community: Field evidence for the novel weapons hypothesis. Journal of Ecology 97: 641-645.

Timper P, Gates RN, Bouton JH (2005) Response of *Pratylenchus* spp. in tall fescue infected with different strains of the fungal endophyte *Neotyphodium coenophialum*. Nematology 7: 105-110.

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. Nature 421: 628-630.

Towne EG, Kemp KE (2008) Long-term response patterns of tallgrass prairie to frequent summer burning. Rangeland Ecology and Management 61: 509-520.

Tracy BF, Renne IJ (2005) Reinfestation of endophyte-infected tall fescue in renovated endophyte-free pastures under rotational stocking. Agronomy Journal 97: 1473-1477.

Transeau EN (1935) The prairie peninsula. Ecology 16: 423-441.

Trappe JM (1987) Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir GR, editor. Ecophysiology of Va Mycorrhizal Plants. Boca Raton, FL: CRC Press. pp. 5-25.

United States Department of Agriculture Natural Resource Conservation Service (USDA NRCS). (2006) State WHIP plan for Kentucky.

http://www.ky.nrcs.usda.gov/programs/WHIP/WHIP2007.html [accessed 28 Jun 2011]

United States Geological Service (USGS) (1981) Geologic Map of Kentucky.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, et al. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396: 69-72.

van Diepen LTA, Lilleskov EA, Pregitzer KS, Miller RM (2010) Simulated nitrogen deposition causes a decline of intra- and extraradical abundance of arbuscular mycorrhizal fungi and changes in microbial community structure in Northern hardwood forest. Ecosystems 13: 683-695.

van Hecke MMV, Treonis AM, Kaufman JR (2005) How does the fungal endophyte *Neotyphodium coenophialum* affect tall fescue (*Festuca arundinacea*) rhizodeposition and soil microorganisms? Plant and Soil 275: 101-109.

Vatovec C, Jordan N, Huerd S (2005) Responsiveness of certain agronomic weed species to arbuscular mycorrhizal fungi. Renewable Agriculture and Food Systems 20: 181-189.

Velazquez MS, Biganzoli F, Cabello MN (2010) Arbuscular mycorrhizal fungi in El Palmar National Park (Entre Rios Province, Argentina) - a protected reserve. Sydowia 62: 149-163.

Vila-Aiub MM, Ghersa CM (2001) The role of fungal endophyte infection in the evolution of *Lolium multiflorum* resistance to diclofop-methyl. Weed Research 41: 265-274.

Vila-Aiub MM, Martinez-Ghersa MA, Ghersa CM (2003) Evolution of herbicide resistance in weeds: Vertically transmitted fungal endophytes as genetic entities. Evolutionary Ecology 17: 441-456.

Vogel JA, Koford RR, Debinski DM (2010) Direct and indirect responses of tallgrass prairie butterflies to prescribed burning. Journal of Insect Conservation 14: 663-677.

Vogelsang KM, Reynolds HL, Bever JD (2006) Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytologist 172: 554-562.

Vos SM, Ribic CA (2011) Grassland bird use of oak barrens and dry prairies in Wisconsin. Natural Areas Journal 31: 26-33.

Washburn BE, Barnes TG, Rhoades CC (2002) Using imazapic and prescribed fire to enhance native warm-season grasslands in Kentucky, USA. Natural Areas Journal 22: 20-27.

Washburn BE, Barnes TG, Sole JD (1999) No-till establishment of native warm-season grasses in tall fescue fields. Ecological Restoration 17: 144-149.

Washburn BE, Barnes TG, Sole JD (2000) Improving northern bobwhite habitat by converting tall fescue fields to native warm-season grasses. Wildlife Society Bulletin 28: 97-104.

Weand MP, Arthur MA, Lovett GM, McCulley RL, Weathers KC (2010) Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. Soil Biology and Biochemistry 42: 2161-2173.

Weather Channel. Undated. Monthly Averages for Lexington, Kentucky. URL http://www.weather.com/outlook/health/fitness/wxclimatology/monthly/graph/USKY1079 [accessed 29 Jun 2011]

Weaver JE, Clements FE (1929) Plant Ecology. New York, NY: McGraw-Hill Book Co.

White DC (1995) Chemical ecology: Possible linkage between macro- and microbial ecology. Oikos 74: 177-184.

Wilkins GR, Delcourt PA, Delcourt HR, Harrison FW, Turner MR (1991) Paleoecology of central Kentucky since the last glacial maximum. Quaternary Research 36: 224-239.

Wilson GWT, Hartnett DC (1997) Effects of mycorrhizae on plant growth and dynamics in experimental tallgrass prairie microcosms. American Botany Journal 84: 478-482.

Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85: 1732-1738.

Wilson GWT, Hartnett DC, Smith MD, Kobbeman K (2001) Effects of mycorrhizae on growth and demography of tallgrass prairie forbs. American Journal of Botany 88: 1452-1457.

Zimmerman J, Higgins SI, Grimm V, Hoffmann J, Munkenmuller T, et al. (2008) Recruitment filters in a perennial grassland: The interactive roles of fire, competitors, moisture and seed availability. Journal of Ecology 96: 1033-1044.

VITA

Sarah Lynn Hall

Birthdate: 12 March 1982 (Richmond, Kentucky)

Education
2005-2006 University of Kentucky (Lexington, KY)
M.S. Forestry, concentration in Restoration Ecology

2000-2003 Appalachian State University (Boone, NC)
B.A. Interdisciplinary Studies, concentration in Environmental Education

Professional Experience
2011-2014 ~ Berea College (Berea, KY)
Visiting Professor of Plant and Soil Science

2007-2010 ~ Kentucky State University (Frankfort, KY)
Co-Investigator of Plant Biodiversity

2004 ~ Institute of Ecosystem Studies (now Cary Institute) (Millbrook, NY)
Research Assistant

2002 ~ Institute of Ecosystem Studies (now Cary Institute) (Millbrook, NY)
Ecology Education Fellow

Honors
University of Kentucky ~ 2011 **Kerri Casner Fellowship** for Travel to Professional Meeting ($1,000) Tracy Farmer Institute for Sustainability and the Environment, 2010 **Kentucky Opportunity Fellowship** ($15,000 + tuition) University of Kentucky Graduate School & Center for Ecology, Evolution and Behavior.

Appalachian State University ~ Summa Cum Laude, Honors Program

Peer-Reviewed Publications
Hall SL and RJ Barney. 2011 (in press). Leaf Beetle (Coleoptera: Chrysomelidae) Biodiversity within Isolated Remnant Grasslands in Kentucky State Nature Preserves. Journal of the Kentucky Academy of Science 72.

Hall SL, RL McCulley and RJ Barney. In press. Restoration of native warm season grassland in a tall fescue pasture using prescribed fire and herbicides. Restoration Ecology. doi: 10.1111/j.1526-100X.2010.00749.x

Barney RJ and **SL Hall**. 2011. New Host Plant Records for Selected Cryptocephaline Leaf Beetles (Coleoptera: Chrysomelidae) in Kentucky. The Coleopterists Bulletin 65(1):15-19. doi: 10.1649/0010-065X-65.1.15.

Hall SL and RJ Barney. 2010. A Quantitative Method for Assigning Abundance Classifications to Leaf Beetles (Coleoptera: Chrysomelidae) in Kentucky. Natural Areas Journal 30(1):95-105. doi: 10.3375/043.030.0110

Hall SL, CD Barton and CC Baskin. 2010. Topsoil Seed Bank of an Oak-Hickory Forest in Eastern Kentucky as a Restoration Tool on Surface Mines. Restoration Ecology. 18(6):834-842. doi: 10.1111/j.1526-100X.2008.00509.x

Barney RJ and **SL Hall**. 2009. *Pachybrachis nigricornis carbonarius* Haldeman (Coleoptera: Chrysomelidae): Abundance, Distribution, and Host Plant Associations with Legumes (Fabales: Fabaceae) in Kentucky. The Coleopterists Bulletin 63(4):467-474. doi: 10.1649/1180.1

Hall SL, CD Barton and CC Baskin. 2009. Seed Viability in Stockpiled Topsoil on a Surface Mine in Appalachia. Ecological Restoration 27(4):381-383. doi:10.3368/er.27.4.381

Hall SL. 2009. Talk of "clean coal" ignores dirty truth. Frontiers in Ecology and the Environment 7(2):70. doi: 10.1890/09.WB.001

Angel PN, CD Barton, RC Warner, C Agouridis, T Taylor, and **SL Hall**. 2008. Forest Establishment and Water Quality Characteristics as Influenced by Spoil Type on a Loose-Graded Surface Mine in Eastern Kentucky. *In* R.I. Barnhisel (Ed.), Proceedings of the 2008 National Meeting of the American Society of Mining and Reclamation, Richmond, VA, June 13- 19, 2008. ASMR, Lexington, KY.

Angel PN, CD Barton, RC Warner, C Agouridis, **SL Hall**, RJ Sweigard and DH Graves. 2007. Tree Growth and Natural Regeneration on Three Loose-Graded Surface Mine Spoil Types in Kentucky: Preliminary Findings. *In* R.I. Barnhisel (Ed.), Proceedings of the 2007 National Meeting of the American Society of Mining and Reclamation, Gillette WY, June 2-7, 2007. ASMR, Lexington, KY.

De Sá PB, J Hartman, W Leesutthiphonchai, AM Holdcroft, P Dymarski, **SL Hall**, JP Royse and J Lensing. 2006. National Nursery Perimeter and Forest Survey for *Phytophthora ramorum* in Kentucky, 2006. Nursery and Landscape Program 2006 Research Report: 30-31.

[Sarah L. Hall]